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AS BIOLOGICAL RESPONSE SYSTEMS TO MONITOR THE NEARSHORE
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DEVELOPMENT OF A METHODOLOGY FOR USE OF
FRESHWATER CLAMS AS BIOLOGICAL RESPONSE
SYSTEM TO MONITOR THE NEARSHORE
ENVIRONMENT OF THE LOWER GREAT LAKES

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ENVIRONMENT OF THE LOWER GREAT LAKES

R. A. C. PROJECT NO. 162 PL

Prepared For
Ontario Ministry of the Environment

NOVEMBER 1988



By

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ENVIRONMENT ONTARIO RESEARCH REPORT

DEVELOPMENT OF METHODOLOGY FOR USE OF FRESHWATER CLAMS
AS BIOLOGICAL RESPONSE SYSTEM TO MONITOR THE NEARSHORE ENVIRONMENT
OF THE LOWER GREAT LAKES

RAC Project No. 162 PL

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Acknowledgement and Disclaimer

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Abstract

We are reporting the results of three years of research on the biological responses of unionid clams, in particular Lampsilis radiata and Elliptio complanata, to variation in environmental factors. WE have approached the question of using clams as indicators of environmental quality considering responses at levels ranging from genetic to community. Research was a combination of observational studies, manipulative field experiments, experiments at the mesocosm level, and laboratory experiments and analyses. We found that shell morphology, degree of shell etching and growth rates vary along a gradient of exposure to water energy. These phenotypic responses to environmental variation appear to have little genetic basis. Few loci are polymorphic, allelic frequencies show little spatial pattern and heritability is low. However, in transplant experiments clams moved to different environments are strongly influenced by the environment from which they came. For example growth rate in the first year and tissue metal burdens at the end of the first year are determined much more by source lake than by destination lake. The "source effect" for growth rate can be explained by (a) early induction due to different environments acting on a common genotype early in life in panmictic populations (e.g., north shore Lake Erie and adjacent waters), or possibly by (b) underlying genetic differences which are a product of selection in amictic populations (e.g., separate lakes on an acidity gradient in the Muskoka/Haliburton region). Our attempts to use contaminant levels in shell as an environmental monitor were not successful. We have had success in demonstrating changes in density and growth rate parameters attributable to pollution, despite the potentially confounding effects of natural environment variation.

TABLE OF CONTENTS

	<u>Page</u>
Acknowledgement and Disclaimer	(i)
Abstract	(ii)
Introduction	1
Results and Discussion	1
(1) The Phenotypic Plasticity of Unionid Clams	1
(2) Distinguishing Responses to Natural Environmental Variation from Pollution-Related Effects	4
(3) Validation of Clam Age from Growth Rings	5
(4) Clam Shell Composition as a Monitor of Environmental Pollution ..	6
(5) Genotypic Basis for Phenotypic Plasticity	8
(6) Influence of Pollution Levels on Clams	9
(7) Predictive Models of Clam Responses	11
Summary and Recommendations	11
Recommendations for Further Studies	13
Reference List for MOE Final Report	14
Appendix 1 - Papers Arising from Research Funded by MOE RAC Project 162 PL	
Appendix 2 - Seminars Presented on MOE-Funded Research	

Final Report - R.A.C. Project 162PL

Title: "DEVELOPMENT OF METHODOLOGY FOR USE OF FRESHWATER CLAMS AS BIOLOGICAL RESPONSE SYSTEM TO MONITOR THE NEARSHORE ENVIRONMENT OF THE LOWER GREAT LAKES"

Introduction: Much of the substance of this final report is contained in the contribution to the MOE Technology Transfer Conference in December 1986 (Green and Hinch 1986). That information can be supplemented by the final year (1987) annual progress report submitted in May 1987, and by the contents of the Ph.D. thesis by Bailey (1987) and the M.Sc. thesis by Hinch (1987). However, there is new information, and citations to other sources, included here as well.

When we submitted our grant proposal in 1984 we had the general objective of investigating the use of freshwater clams as "response systems" for use in environmental studies. The following specific objectives were necessarily associated with that general objective: (1) to assess the phenotypic plasticity of unionid clams when subject to variable environmental conditions, (2) to assess whether responses to natural environmental variation can be distinguished from "pollution" effects, (3) to validate the aging of clams from shell growth rings, (4) to assess whether chemical composition of shell (especially in "ageable" layers of shell) can be related to pollution that occurred during the life history of the clam, (5) to assess the genotypic component of the phenotypic response of clams to the environment (with emphasis on larval development and heritability of morphological ontogeny), (6) to evaluate the influence of known pollution situations on growth rate, shell morphology, shell chemistry and soft tissue chemistry, and (7) to build models which predict the responses of clams from the properties of the natural environment and permit tests of hypotheses related to the additional effect of pollution-associated factors.

I think we have done a productive and generally successful three years of research. We have achieved most of the specific objectives - at least to our satisfaction. For those objectives we have not achieved, we now see the path to achieving them more clearly and can better design research for that purpose.

Results and Discussion: This section will be organized by the list of specific objectives given in the Introduction.

(1) The Phenotypic Plasticity of Unionid Clams in response to varying natural environmental conditions has been inferred by many malacologists who have described intra-specific variability of shell form as a function of various environmental factors. However their concern has been taxonomic rather than environmental; e.g. a key which uses ratio of width to length as a species identification character may fail if the shape of a species varies. Some of our research previous to 1984 (when this grant period began) dealt with the description of variability in shell form of a species as a function of varying chemical and physical environment (Green 1972, Bailey et al. 1983). Our research supported by the MOE grant has focused on two unionid species, Lampsilis radiata (studied mostly along the Lake Erie north shore) and Elliptio complanata (studied mostly in "cottage country" lakes of the Muskoka, Haliburton and Algonquin Park regions). Both are abundant and widespread species, eminently suitable for monitoring for environmental quality. Some

previous studies on those genera, related to response to environment, are: Green 1971, 1972; Smith et al 1975; Anderson 1977; Green 1980; Heit et al 1980; Leard et al 1980; Tessier et al 1984; Dermott and Lum 1986; Czarnzki 1987; Servos et al 1987.

Studies by Bailey in 1984 showed that the unionid community of Inner Long Point Bay, Lake Erie, is diverse (10 species collected) and that the species composition varies, with the dominant axis of change coinciding with the gradient of exposure to wind and waves - from the open lake (outer Long Point Bay) to the shallower sheltered interior of the inner bay. Descriptive multivariate statistical methods were used to describe the axis of community composition change, and to relate it to the environmental gradient. These results have been reported by Bailey (in press). Morphological variation in the shell of one species, Lampsilis radiata, was then studied. Again there was a gradient of biological response coinciding with the "exposure gradient". Shells of L. radiata had heavier shells at the high exposure end of the gradient. During 1984 a reciprocal transplant experiment was done in the field by Hinch and Bailey (Hinch's honours project), involving large numbers of marked clams moved or left as controls for most of the open water season. Again the clams showed faster growth at the more exposed site. There was also an effect related to the source (low vs. high exposure sites) of the transplanted clams. These results have been reported by Hinch et al (1986).

To investigate the possible adaptive significance of shell form regarding efficiency of burrowing in sand (at high exposure sites) versus mud (at low exposure sites), Bailey transplanted marked clams from high and low exposure sites in Inner Long Point Bay, into large experimental ponds containing mosaics of mud and sand substrates. After ca. 2 months the clams were collected and their "choices" of substrate noted. Clams from both high and low exposure sites in the Bay showed a preference for mud, perhaps because they move less easily in mud. These results will be reported by Bailey (paper in preparation). Finally, an experiment was done in the laboratory to determine whether shell shape has adaptive value in burrowing. Bailey placed L. radiata of different shapes onto mud and sand substrates and recorded the length of time required for each clam to re-bury itself. He found that narrow mussels were more apt to burrow in the sandy substrate than obese mussels, but this was not true in the muddy substrate. Heavy-shelled individuals were less likely to burrow in the sandy substrate. These results are in general agreement with Stanley (1970) who suggested that less obese shells are better adapted to burrowing in hard substrates, and that thinner shells enable bivalves to burrow faster. However these results are not in agreement with Bailey's observations on the natural population, where clams in the exposed, sandy areas tended to have heavier shells, and no relationship between obesity and substrate was seen. Hinch et al (1986) did observe that shells were less obese in exposed, sand habitats. These results will be reported by Bailey in a future paper.

Studies on the phenotypic plasticity of the unionid clam Elliptio complanata in response to varying natural environmental conditions were conducted by both Hinch and Green. From his honours project work with Bailey on L. radiata in Long Point Bay, Hinch moved on to M.Sc. studies on E. complanata in lakes of the Muskoka/Haliburton region. These are lakes threatened, in varying degree, by acidification. Before assessing effects of acidification on the clams, Hinch conducted several studies to evaluate effects of natural environmental variation - especially exposure to water turbulence - that might be confounded with effects of water chemistry change driven by

acidification. First, in 1985, Hinch re-sampled 60 lakes which had been surveyed by MOE. Emphasis was put on collection of *E. complanata* (or determination that they were not present) but water chemistry analysis was also done. These data are now being analyzed and will be the basis of a future paper. Clams were found in 40 of these lakes. Four of these lakes (Green, Beech, Bark and Tock) were selected for intensive study. Selection was based on similarity of lake morphology, ease of access, and coverage of a range of alkalinity and pH (pH: 7.5, 7.2, 6.3 and 6.0; alkalinity: 243, 238, 153 and 22, respectively). Also in 1985 clams were collected from a site on the lee shore and a site on the windward shores of these lakes, and shells were compared among lakes (representing water chemistry effects), between sites within lakes (representing effects of exposure to water turbulence), and among clams within sites. Far more of the variation in shell form (including size variation) was due to between-site exposure differences than to among-lake chemical differences. These results have been reported by Hinch and Bailey (in press). Extent of shell erosion, or etching, was also studied. A microcomputer-based digitizer system was used to quantify the patterns of eroded periostracum and nacre. Shells of clams from high exposure sites were proportionally more etched than were shells of clams from low exposure sites in the same lakes, suggesting that etching is a physical process related to water turbulence. Etching was not related to among-lake variation in water chemistry. These results have been reported by Hinch and Green (submitted, in review).

During 1986 Green began studies (in collaboration with Ron Hall of MOE) on the relationship between Baptiste Lake *E. complanata* shell growth rate and form, on the one hand, and physical/chemical environment and infaunal productivity, on the other hand. Baptiste Lake is a large lake (22.3 km²) of the Algonquin Park region, in contrast to the small (average area 1.5 km²) Muskoka-Haliburton lakes studied by Hinch. The preliminary research in 1986 involved SCUBA sampling around a small (ca. 0.2 ha) island which is exposed to ca. 4 km of open lake on its windward shore but protected by nearby land on its leeward shore. The shells of all clams were measured, weighed and sectioned for age determination. Sediment samples were taken at the location where each clam was collected, and depth was recorded. Emergence traps were set overnight by Ron Hall. The sediment samples are now being analyzed for particle size distribution and also for infaunal organisms. Ron Hall is assisting with identification of invertebrates, both infaunal stages and emergent stages. The results indicate that shell size and shape are influenced by exposure to water turbulence (in a manner similar to that found by Hinch). Based on the age determinations, growth rate is higher on the more exposed shore. However age distribution does not systematically differ between the exposed and protected shores. These results will be written up for publication. They were presented at a paleoecology conference held at UWO this September.

In summary, we have evidence of substantial intra-specific phenotypic plasticity in shell growth rate and form when clams are subject to different natural environmental conditions. Exposure to water turbulence appears to be a particularly important factor, so that any study conducted for the purpose of assessing clam response (e.g., growth rate or shell form) to pollution (e.g., acidification) must carefully control for exposure to water turbulence - whether it be wind-generated turbulence in lentic environments or current in lotic environments.

(2) Distinguishing Responses to Natural Environmental Variation from Pollution-Related Effects: The above discussion suggests that it may be difficult to distinguish effects caused by natural environmental variation from pollution-related effects, at least regarding responses such as growth rate and shell form. Even in small lakes the difference between lee and windward shorelines appears to be much more important than does among-lake differences in pH covering the range 6.0-7.5 (a ca. 3-fold range in hydrogen ion concentration) and alkalinity covering the range 22-243. Results from a one-year 3-lake reciprocal transplant experiment (Hinch M.Sc. thesis research) has shed additional light on the feasibility of using E. complanata as monitors of acidification and heavy metal concentrations when exposure to water turbulence is held constant. Growth in the first post-transplant year was not related to the acidity of the lake to which the clams were transplanted, but was strongly related to the source lake (in which the clam had spent its previous life). This suggests a strong "carryover" effect. Metal burdens after the first post-transplant year were also most strongly related to the source lake, as opposed to the destination lake.

An earlier study on L. radiata (Green 1972) found that different aspects of shell morphology responded to an environmental factor interpreted as water turbulence, as opposed to another environmental factor which was interpreted as water chemistry. Stated simply, proportional shell thickness varied with water chemistry whereas overall shell size and extent of shell sculpture varied with water turbulence. Such a result suggests that response to a natural environmental factor such as water turbulence may be distinguishable from a pollution-related effect on water chemistry (e.g., pH and alkalinity). A similar result was found in these studies. In his 40-lake comparison of shell form Hinch found a factor indicating that longer, thicker shelled clams were related to increasing alkalinity, conductivity and pH. Almost as important was another factor which indicated that larger, more streamlined clams were related to more turbulent environments.

It may be that growth rate and shell form are poor responses to use if one wishes to distinguish between natural environment variation and change caused by pollution, despite (in a sense because of) the sensitivity of those responses to the environment. What of other responses, ones that are more directly linked to types of pollution? For example, one would think that elevated levels of heavy metals in shell or in tissue should not be influenced by increased water turbulence. However other studies (refs. cited in Hinch and Stephenson 1987) have shown that, all else being equal (e.g., ambient environment metal concentrations and pH), the bioconcentration of metals by clams is influenced by their growth rate; thus if clams grow faster where there is more water movement it follows that metal concentrations in shell or tissue may differ even when concentrations in the ambient environment are the same.

In any case there is the philosophical distinction between a response which indicates a change in physiological health (e.g., decreased growth rate) and a response which just substitutes a clam for another sampling device (e.g., chemical concentration in tissue or shell). The former is to be preferred. Also it turns out that there are severe technical problems associated with chemical analysis of shell (see section 4 below), so that concentration in soft tissue is the viable option at present - and that may provide a transient rather than a time-integrated picture of the pollution situation. As Hinch and Stephenson (1987) found, and Smith et al (1975) had found previously, the

concentration of metals in tissues does usually have some relationship to ambient metal concentration and to pH and alkalinity, but the relationship is not usually a simple one. It depends on the particular tissue, the particular metal and other factors. (See section 6 below.) Why not just do water and sediment chemical analysis for the heavy metals instead? After all people do not usually eat freshwater clams or eat the other species that do eat them (e.g., raccoons, freshwater drum).

What about genetic response? This is covered in section 5 below, but it might be noted here that Nevo and co-workers (Nevo et al 1984, Baker et al 1985) have published evidence of selection for alleles at particular loci by particular heavy metals, in marine shellfish. We are proposing future research related to this question.

(3) Validation of Clam Age from Growth Rings: We now feel confident about our ability to age the unionids Lampsilis radiata and Ellptio complanata using annual growth rings in the shell, at least for lentic populations occurring at the latitudes of north shore Lake Erie and south-central Ontario. A thin section showing annual rings of L. radiata is in McCuaig and Green (1983). The temptation to skip the work of preparing thin sections should be resisted. Agreement between age estimates obtained from examination of external shell bands and those obtained from examination of thin sections is not good, and in most large specimens the older (outermost) bands are unreadable on the shell exterior. We estimated ages for specimens of three species of unionids from Atlantic Canada, in a collaborative study with Janice Metcalfe of Environment Canada (Canada Centre for Inland Waters). In the report of that study (Metcalfe et al 1987), the three unionid species are compared, different sites of collection are compared, the results of two different people who made independent estimates are compared, and for one species the results of estimates from external versus internal rings are compared. This study is discussed further in section 4 below.

Annual rings are usually clearer in freshwater than in marine species because there is no shell structure induced by tidal cycles. They are clearer in lentic populations than in lotic populations of freshwater clams because there is no shell structure induced by variable flow rates. Some unionid species are easier to age than others, and L. radiata and E. complanata are two of the easiest. Therefore it should be kept in mind that in our MOE-funded studies we have been working with the optimum situation for aging clams using their shell annual rings.

We have used three different approaches. One, the Walford Plot technique, is described and used in Green et al (1983) and in McCuaig and Green (1983). Here the external rings are measured, the lengths of the clam at two successive annual rings are plotted against each other, and the size-vs.-age growth curve is derived (for a sample of clams). Thin sections of shells of some or all of the clams are also prepared and examined to verify that the measured external rings are really annual rings. The advantage of this approach is that absolute ages of two rings need not be known, only that they are two consecutive years. The disadvantages are that external shell rings must be readable to some extent, and also that one does not directly obtain an age estimate for an individual clam (although one can do so indirectly by back-calculation from length). The second approach is to prepare thin sections of the shells of all the clams to be aged, and count the number of internal bands (preferably several independent counts by different people). Direct counts of annual rings

were done for the three unionid species from Atlantic Canada, as mentioned above; for L. radiata in the Long Point Bay study; for E. complanata in the Muskoka-Haliburton lakes study; and in the Baptiste Lake preliminary research. This approach produces an age estimate for each clam, but is sensitive to missing annual rings on the one hand or false rings on the other - that is, obtaining the correct age at a given ring depends on an accurate count up to that ring. If the count for a given ring is incorrect then all "later" rings will be incorrect as well, and the cumulative error can be large for older clams. A third approach is to collect clams, measure and mark them and return them to the habitat for at least a full growing season - preferably for a full year. Then they are collected again and re-measured, yielding size-specific growth rates from which the size-vs-age growth curve can be calculated. If the clams are returned to the habitat for exactly a year then the Walford Plot method can be used to derive the curve. This method is the most accurate - for that given year. But that year may be an unusual one, and the clam may be disturbed by the collection, handling and return to the habitat. Also no direct age estimate is obtained for each individual clam.

In summary, all three methods have their strengths and weaknesses. The wise researcher will use all three, or however many are possible, when he can. Good agreement among age estimates, or estimated growth curves, obtained by different methods is the most convincing evidence. We have used methods 1 and 2 for ageing a clam population whenever external annual rings seemed readable. Use of method 3 in association with the reciprocal transplant experiment done on L. radiata in Inner Long Point Bay was limited by the fact that the clams were only returned to the habitat for ca. 4 months (June - October), and the proportion of annual growth represented by that period of time is uncertain. Hinch's one-year 3-lake reciprocal transplant experiment, mentioned in section 2 above, should provide an opportunity to use all three methods on E. complanata in control groups (clams not moved between different lakes).

(4) Clam Shell Composition as a Monitor of Environmental Pollution: At the beginning of the three-year grant period, we placed heavy emphasis on research to assess the potential for using chemical composition of shell as an indicator of chemical pollution of the environment in which the clam lived and formed its shell. The results were disappointing, though not out of line with what others have found when they went beyond a superficial assessment.

Much of the technical work on this problem was done by Cynthia Walker, who was my technician until February 1985. Her background included extensive training (both as a student and as an employee) in analysis for heavy metals at the Trent University environmental studies program. We found that in practice she was often showing the UWO Geology Dept. Faculty and students how to best use their own analysis equipment (e.g., atomic absorption spectrophotometer). Besides the analyses that she conducted herself, Cynthia Walker also trained other people in my lab - such as Scott Hinch - to do analyses for heavy metals. Financial support was from three different sources: Environmental Protection Service of Environment Canada (Edmonton) re. detection of effects of an oil spill in shells of arctic marine species; National Water Research Institute of Environment Canada (CCIW, Burlington) re. detection of mercury and arsenic in shells of three unionid species from habitats polluted by mine waste in Nova Scotia (see section 3 for previous reference to this study); and the Ontario M.O.E. grant support re. detection of contaminants in unionids generally.

Besides the paper submitted to the M.O.E. Technology Transfer Conference

(Green and Hinch 1986), our results of these studies are reported in Metcalfe et al (1987). A summary follows.

A number of workers have recently suggested that analysis of contaminant concentrations in shell holds great promise and may offer several advantages over the use of soft tissues (Imlay 1982; Koide et al 1982; Fang and Shen 1984; Carell et al 1987). Shells may retain a record of contaminant levels over many years, and the concentration factor (shell concentration relative to ambient environment concentration) may be greater than for soft tissue. Correlations with pollution may in some cases be higher (e.g., Koide et al 1982). Other workers, however, have found problems in analysis and problems in interpretation of results (Lutz 1981, Dermott and Lum 1986, our results, and others). We have the impression that in this area of research there is a strong negative correlation between (a) enthusiasm for the potential of contaminants in shell as a reliable monitor of past and present environmental pollution and (b) the amount of research that had been done on the subject and the depth of experience acquired.

The problems can be divided into three categories. First, there are analytical problems per se, such as low contaminant concentrations, calcium interference, and similar. Second, there are problems related to interpretation of results because of the influence of factors other than the ambient environmental concentration of contaminants or the degree of acidification. The age and size of the clam, its growth rate at the time of the impinging contamination, change of the concentration of the contaminant in the shell after initial deposition (due to diagenesis for example), and metal-specific behaviour in deposition can all affect the result obtained. For example, Dermott and Lumm (1986) found in a study on Elliptio complanata that different metals behaved differently in deposition near a pollution source, in chronological trends within the shell prismatic layer, in relationship with clam age, and in relationship with times of fastest growth rate. Third, there are problems directly related to the attempted use of chronological layers laid down in shell for the purpose of reconstruction of an environmental contamination history (over the life span of the clam). This is one of the more attractive possibilities where unionid clams are involved, because most species live for at least a decade, and some live for two decades or longer. Unfortunately one is usually reduced to a very small sample of shell to analyze if one wants to do separate analyses, annual layer by annual layer. Furthermore, those techniques which do not require separation of the shell into annual layers before analysis tend to be less sensitive or less reliable, whereas the methods commonly used to separate annual layers can easily change the contaminant concentrations.

These problems are also discussed extensively in Green et al (1985).

We analyzed shells from several sources, including unionids from Lake St. Clair (courtesy of P. Hebert and C. Pugsley, U. of Windsor) and from mine waste contaminated Nova Scotia lakes (Metcalfe et al 1987). In both cases we know contaminant (metals) concentrations in sediment and in clam soft tissues; they were high. Our attitude was that we should be able to detect the metals in shells of clams from such polluted sediments, or there was no point in trying to do so for monitoring in slightly polluted environments. Our results were similar for clams from all sources - here I will concentrate on the Nova Scotia material. Specimens of Elliptio complanata and Anodonta imbecilis were used, and analyses were done for As, Hg and Pb, which were in high concentrations in soft tissue and were known pollutants in the habitats concerned. The electron

microprobe (EM) and the more sensitive secondary ion mass spectrometry (SIMS) were used as "step scan" probe techniques which could estimate elemental concentrations in different shell layers across the edge of a polished thin section of shell. The EM has a sensitivity of about 200 ppm whereas SIMS can achieve a detection limit as low as 1 ppb for some elements. No reliable estimates were obtained with the EM because of its poor sensitivity. With SIMS it was possible to detect these elements using extended counting periods, but variability was high. This method is time-consuming and very expensive. A number of improvements to the technique may be possible (see Metcalfe et al 1987 p.26) but we did not pursue it. If specially prepared standards are used that are appropriate for calcified tissue, with special sample holders to control surface charge-up, and interferences are somehow reduced, then this method may prove useful.

We also tried atomic absorption mass spectroscopy but calcium interference proved to be a major problem. Digestion to remove calcium but not change metal levels was difficult. We applied all methods to known standards as well as to test material. Attempts were made to separate shell layers before AA spec analysis, using heat shock and also using oxidants, but apparently this drives off metal-containing material (perhaps organic constituents are oxidized), because the sums from the layers did not add up to the total for unseparated shell layers. Little is known about how contaminants are bound within the calcium carbonate matrix (Koide et al 1982, Fang and Shen 1984, Swann et al 1984).

In the end, we decided that the state of the methodological art had to advance significantly before analysis for contaminants of shell would be a useful tool for pollution studies. It seemed that such advances would probably occur in geological or nuclear chemistry laboratories, and that our efforts were better put elsewhere. For example, we were aware that Dr. Michael Risk's lab in the Geology Dept. at McMaster University was working on the calcium interference problem. Carell et al (1987) describe impressive results obtained using a new analytical technique, the proton microprobe, which appears to combine the step-scan capabilities of the EM and SIMS with high resolution and reliability. We await further validation of these new methods with great interest.

(5) Genotypic Basis for Phenotypic Plasticity: To what extent is there a genetic basis underlying the phenotypic plasticity of response of unionid clams to varying environmental conditions? Does the genotype, as well as the phenotype, track environmental change?

Most of the research on this aspect was done by Robert C. Bailey, in pursuit of a Ph.D. which he was awarded in May 1987. The information is in his thesis but several papers are also in preparation. The unionid species studied was Lampsilis radiata, mostly in Long Point Bay, Lake Erie.

Morphometric and annual ring analysis of L. radiata from Inner Long Point Bay showed that individuals from more exposed, sandier areas were faster growing and had thicker shells (Bailey and Green in press). Could this spatial phenotypic variation have a genetic basis? Electrophoretic analyses indicated little divergence in frequencies of allozymes at the two gene loci PGM and PGI over the area of the Inner Bay. Other enzymes were checked but proved to be monomorphic (MPH), not detectable (AO, LDH, G6PDH), or poorly resolved (LAP, HEX). All these results suggest low genetic variability. Moving to a larger geographic scale, there were no differences in genotype frequencies between

Long Point Bay L. radiata and ones from Lake St. Clair and the Detroit River (data of P. Hebert, Univ. of Windsor). Only when one looked as far afield as Balsam Lake on the Trent-Severn waterway were genotypic differences from the Long Point Bay population found at the PGI locus (fixation on the slow PGM allele). There were some differences in frequencies at the PGM locus between areas of differing exposure in Inner Long Point Bay (fewer heterozygotes at lower exposure), but at a very low level. Certainly reproductive isolation within areas of the Inner Bay would not be a sustainable hypothesis. In two transplant experiments (on L. radiata between sand and mud in Inner Long Point Bay, reported in Hinch et al 1986; and on E. complanata among three lakes of differing water chemistry in the Muskoka-Haliburton area, reported in Hinch's M.Sc. thesis) a "source effect" was important in predicting growth rate in transplants. That is, how clams grew was in part predictable by where clams were transplanted from. Superficially this suggests some genetic property, but it is also explained by early induction: early determination of expression of genes in a common genotype by the influence of particular local environments. Thus the growing clam adapts, grows in a manner typical of that environment, and continues to do so even when transplanted later in life to a different environment.

Bailey investigated heritability of size and shape in glochidia (parasitic larvae) of L. radiata by comparing glochidial morphological variation among and within families (50 glochidia from each of 15 female L. radiata). Heritability was estimated to be 10-19 percent, which is low, about the same as for heritability of litter size in mice and egg production in poultry, but much lower than for plant height in corn or stature in humans (65-70 percent). Heritability of larval size in marine bivalves can exceed 50 percent (reviewed in Newkirk 1980).

In summary, variation in morphology and growth rate of L. radiata appears to be largely phenotypic plasticity, unrelated to adaptation in the sense of any genotypic basis. However, it should be noted that we have only been looking at natural environmental variation, and that chronic point source pollution may indeed produce a genetic response. Nevo and co-workers (Nevo et al 1984, Baker et al 1985) have published evidence of natural selection for increased resistance to mercury pollution in a marine mollusc. Allozymes at the PGM and PGI loci were involved, and these are loci which we have shown to be polymorphic in L. radiata. We are proposing further research related to this question, which will be based on field and laboratory genetic studies of unionid clams from one or two chronic point source heavy metal pollution sites in Ontario.

Additional information on genetic bases for response to pollution will come from electrophoretic analyses which will be done in early 1988 on E. complanata which were involved in the 3-lake reciprocal transplant study carried out by Scott Hinch, in relation to lake acidification and heavy metal uptake. Both growth rate and metal burden of soft tissues showed a "source effect" in the transplant experiments. See sections 2, 5 and 6 for more information.

(6) Influence of Pollution Levels on Clams: Studies of the influence of known pollution situations on growth rate, shell morphology and soft tissue chemistry have been carried out by Scott Hinch. He successfully defended his M.Sc. thesis in early December 1987. His studies were on Elliptio complanata in Muskoka-Haliburton lakes which

varied widely in their alkalinity and their vulnerability to acidification. (See also sections 1, 2 and 5.)

As previously noted, one must carefully control for variation in exposure to water turbulence or that factor will outweigh the influence of water chemistry on growth rate. Hinch found this to be true, as did Green in recent studies on E. complanata in Baptiste Lake. Data from 40 lakes containing E. complanata that had previously been surveyed by MOE (see section 1) were analyzed for patterns in growth rate and shell morphology, and showed that larger and thicker shelled clams were related to increasing alkalinity, conductivity and pH. A separate relationship with coarseness of substrate and exposure was also found (more streamlined clams in coarser substrates).

In a comparative study contrasting the clams in Tock (alkalinity 22, pH 6.0) and Beech (alkalinity 238, pH 7.2) lakes, Hinch and Stephenson (1987) found that metal concentrations in the clams reflected the levels in the lakes. That is, absolute levels of Cu, Cd, Zn and Mn are greater in Tock, but Mn and Zn levels relative to Cu and Cd are greater in Beech. These differences are consistent with other information about the biological interactions of metals and effects of acidification. Beyond this generality the concentrations of metals were a complex function of tissue type, of the particular metal, and of the size and the age of the clam. In other words, the story is far from a simple one. Concentrations in gill were often twice that in the body. Both size and age of clam generally contributed to metal concentration, but the relationship varied with tissue type and metal.

A large-scale reciprocal transplant experiment was done among Beech, Bark and Tock lakes (Bark: alkalinity 153, pH 6.3) in fall 1985, with controls (clams handled, marked and measured as were transplanted clams, but returned to the same site in the source lake). Degree of exposure to turbulence was held constant. A year after the transplants, the clams (including the controls) were recovered, with an 85 percent recovery rate. Growth over the year was determined, as were concentrations of selected metals. The results may be summarized as follows.

The pollution situation to which the clams were introduced (the three different lakes covering a 6.0-7.2 range of pH and a 22-238 range of alkalinity) had little effect on either the first post-transplant year of growth or on the soft tissue metal burdens as measured at the end of the first post-transplant year. Since metal analysis is destructive, there is no information about pre-transplant metal levels for these clams; however we have metal levels for the control clams. Any "destination lake" effect, or "source-x-destination interaction" effects, were small relative to the "source lake" effect and involved Bark Lake which was intermediate in water chemistry between the other two lakes and therefore can not represent an acidity effect. The implication of this reciprocal transplant experiment appears to be that when environmental biologists transplant clams in order to monitor sites that are possibly polluted, they should be aware that the clams' subsequent growth and metal burdens may be heavily influenced by the clams' previous history of environmental influences.

Bailey and Green (submitted) report density and age-structure changes over the period 1972-1986 in a population of the unionid Anodonta grandis, changes which may be caused by hydrocarbon pollution. This is based on re-sampling by Bailey in 1986 of a population that Green (1980) studied in 1972.

(7) Predictive Models of Clam Responses: Our last objective was to build models which predict components of variation due to natural environmental variation, and to pollution, thereby permitting the predictive distinction of the one from the other.

We have achieved this in some regards. For example, Hinch and Bailey (in press) have derived a components of variation ANOVA model (Model II ANOVA) which quantifies the growth and morphological variation attributable to exposure as opposed to among-lake differences (surrogate for differing water chemistry). Hinch and Green (in review) did the same re. extent of shell etching (erosion). Green has derived a predictive model which accounts for the influence of exposure on growth rate and shell morphology around the edge of a small island in Baptiste Lake. Metcalfe et al (1987) derived growth models for 3 species of unionids at two Nova Scotia sites differing in degree of pollution by As and Hg. Growth rate for Elliptio complanata was significantly lower at the more polluted site.

Summary and Recommendations

We have approached the question of using freshwater clams as indicators of environmental quality on a broad front, considering responses at levels ranging from genetic and physiological to population and community. Research was a combination of field observational studies, manipulative experiments in the field (reciprocal transplants), experiments at the mesocosm level (experimental ponds), and laboratory experiments and analyses. State-of-the-art technology was employed throughout, from SCUBA techniques in field work, laboratory culture for larval development (of glochidia whose culture has only been possible for about a decade), electrophoresis and microelectrophoresis for allozyme determinations in genetic studies, the most modern analytic techniques for metal and isotope analysis in tissue and shell, to sophisticated statistical techniques for describing multivariable responses. Good experimental and statistical design was used throughout. Ph.D., M.Sc., and summer NSERC projects were supported, and all completed successfully. At this point, three years from the start of the grant, 5 papers presenting results are in print (not counting a Ph.D. and an M.Sc. thesis), 3 are in press, 4 are in review, and 12 presentations at meetings and conferences have been given. Probably 4 or 5 more papers presenting results from this work will be published. Promising research to continue along two lines is indicated.

We think this is a credible record of achievement related to the 3 years of MOE grant support which we gratefully acknowledge.

There are a number of practical consequences and recommendations arising from our results:

1. Natural variation, e.g. in growth rate, shell morphology, contaminant levels in shell and soft tissue, can easily mask responses to pollution. The natural variation may be among individuals at the same location, due to differences in age, size, etc., or it may be among habitats in the same lake (e.g. differing in exposure to turbulence), or it may be among habitats in the same lake (e.g. differing in exposure to turbulence), or it may be among lakes for reasons unrelated to the pollution gradient (e.g., the "oddness" of Bark Lake in our studies). Unless the design of studies and the statistical analyses are carefully planned, the results obtained may be uninterpretable or - even worse - misleading. Designs must be balanced with respect to clam ages and sizes, and with respect to exposure to turbulence and the resulting substrate type differences. The

- only safeguard against odd lakes influencing results is to include enough lakes in the study to diminish the influence of an odd lake, and if possible to use "among lakes within a pollution level" as the error term in any tests of pollution effect. This would avoid pseudoreplication and ensure that an odd lake would have a conservative effect (reduce the likelihood of obtaining a significant pollution effect) rather than a liberal one (falsely causing a significant pollution effect).
2. The way a clam responds to a transplant into a new environment appears to be very heavily influenced by the environment it had lived in for all its life before it was transplanted. Therefore any attempt to monitor an environment using introduced clams should, at the least, obtain all clams from the same habitat in the same lake (and preferably clams of the same age and size). However, even if this is done it may not be possible to compare studies in which clams were obtained from different sources.
 3. Some unionid clams can be aged reliably from shell annual rings (e.g., Elliptio complanata, Anodonta spp.), but older individuals (>ca. 10 yr) are more difficult to age with accuracy. Estimates of age or of size vs. age curves should never be based on shell external rings alone. At least some shells should be sectioned, and internal bands examined, to validate the external "annual" rings. If possible, age estimates from external rings and/or counts of internal bands should be validated by a mark-recapture experiment.
 4. The use of mollusc shell as a record of contamination history is an idea whose time has not yet come. There are promising new techniques on the horizon, but even if they solve the current analytical problems there will still be problems in interpretation caused by variation in shell contaminant concentrations due to growth rate, time since deposition, contaminant-specific deposition processes, etc. Simplistic interpretations of contaminant concentrations in shell should be avoided, and should be accepted with caution when it is the work of others.
 5. Allelic frequencies do not appear to vary in any important way in response to environmental variation. Therefore the environmentally determined phenotype (e.g., in shell size and form) that we see is probably based on a phenotypic plasticity not determined by a varying genotype. This may in fact be good news, because (a) we do not have to worry much about genetic determination when predicting environmental quality from clam phenotype, and (b) if we do find loci which have alleles that respond to particular pollutants then such a response will not be masked by high genetic variation caused by natural environment.
 6. Metal burdens in soft tissue are roughly indicative of the concentrations of those metals in the lake, but there are complex relationships with tissue type, what the particular metal is, clam age and size, and probably other factors as well. In addition, if the clam is a transplant from another lake, then its metal burden will probably reflect the source lake more than the destination lake for an uncertain time period (at least a year).
 7. Predictive models for environmental monitoring can be usefully based on responses by unionid clams. Two approaches are suggested. General quality of environment, integrating all influences whether natural or pollution-related, can be predicted from age-specific growth rate and shell morphology. Pollution effects attributable to a particular type of pollution may be predictable using locus-specific allelic

responses at the genetic level. Research to develop these two approaches is planned.

Recommendations for Further Studies: However, despite these achievements, we feel that this is more an area for further research and development of models than it is an area where we can rest on our laurels. We have done much necessary groundwork, establishing the levels of natural variation at various levels - in growth, in shell morphology, in tissue metal burdens, and in allelic frequencies. Now we propose to continue along two lines. One is to accept that the most sensitive response by unionid clams to environmental quality (in the sense of suitability for growth and for high levels of secondary production) is growth rate, which is easily measured from shell annual rings. We propose to build a model which predicts benthic secondary production (emphasizing infaunal species important to fish) from clam growth rate parameters, which will be demonstrated to relate to exposure, depth, water chemistry, and other environmental factors. Eventually intensities of selected types of pollution will be included. Initially this project will continue from the Baptiste Lake work already under way, probably involving a Ph.D. student, and then it will extend to other lakes as well.

This first line of approach is one which in effect abandons the search for the "silver bullet", the biological response which indicates a particular kind of pollution. Instead it focuses on a response which integrates all influences and indicates whether, all natural and pollution-related environmental factors considered, the clam is "happy". If it is growing fast and converting food into tissue and shell at a high rate then presumably the mix of influences on it constitutes a good mix, and presumably it is a good mix for useful secondary production in general (not just by the clam population). But of course this does not permit a backwards-calculation to what the intensity of a particular kind of pollution is. The second line of approach is to seek the silver bullet at the genetic level, to follow up on the idea of Nevo and co-workers that there is a genetic response to metal pollution that may be quite specific. Here we would build a model which predicts a particular shift in the frequency of allozymes at a particular locus, such that when we see that shift it would be evidence of that particular kind of pollution. We would proceed in stages. One or two chronic point-source pollution sites involving heavy metals would be chosen. (Sites suggested by Peter Kauss of MOE have already been visited.) Clams above and below the source would be analyzed for metal burdens, and then larger numbers would be analyzed electrophoretically for allelic frequencies at selected loci. Any above-below differences would be followed up by laboratory selection experiments (differential survival by genotype under metal pollution stress).

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APPENDIX 1

PAPERS ARISING FROM RESEARCH FUNDED BY M.O.E. R.A.C. Project 162-PL

- 1) Bailey, R.C. 1987. Variation in the shell morphology and growth rate of Lampsilis radiata, a freshwater mussel. Ph.D. Thesis. University of Western Ontario, London, Ontario.
- 2) Bailey, R.C. (in press). Correlations between species richness and exposure: freshwater molluscs and macrophytes. *Hydrobiologia*.
- 3) Bailey, R.C., and R.H. Green (in press). Within-basin variation in the shell morphology and growth rate of a freshwater mussel. *Can. J. Zool.*
- 4) Bailey, R.C., and R.H. Green (submitted). Temporal changes in a freshwater mussel population: a natural experiment. *Can. J. Fish. Aquat. Sci.*
- 5) Green, R.H., and S.G. Hinch. 1986. Freshwater clams as monitors of variation in environmental acidity and trace metal levels. In Proc M.O.E. Technology Transfer Conference, Part D, Analytical Methods, pp.189-214. Toronto, Ontario.
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- 7) Hinch, S.G. 1987. Acidification effects on shell morphology and trace metal uptake in the freshwater clam Elliptio complanata. M.Sc. Thesis University of Western Ontario, London, Ontario.
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VARIATION IN THE SHELL MORPHOLOGY
AND GROWTH RATE OF Lampsilis radiata,
A FRESHWATER MUSSEL

by

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of the requirements for the degree of
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ABSTRACT

I measured a correlation between the habitat of a freshwater mussel, Lampsilis radiata siliquoidea (Barnes 1823), and its shell morphology and growth rate. I then tried to determine the source of morphological and growth rate variation (environmental or genetic), and whether or not the phenotype/habitat correlation was due to differential adaptation.

Morphometric and annual ring analysis of mussels from Inner Long Point Bay, Lake Erie indicated that L. radiata from more exposed, sandier areas of the bay were faster growing and had thicker shells than those from less exposed, muddy areas. Variation among exposure areas in allozyme phenotypes of two gene loci (PGM and PGI) showed little evidence of genetic divergence. Statistically significant heterogeneity among exposure areas in PGM genotypes was small relative to that between mussel populations in Balsam Lake (in the Trent-Severn watershed) and the lower Great Lakes. Heritabilities of glochidia shell dimensions were low (<20%), providing little evidence of past or present disruptive selection of shell dimensions. Substrate preference was displayed by the mussels in experimental ponds. L. radiata preferred a finer, more heterogeneous substrate over a coarse sand, and larger mussels showed a stronger substrate preference than smaller individuals. Such habitat preference could influence the breeding structure of the population near the

borders of habitat areas.

A laboratory experiment using L. radiata from Inner Long Point Bay showed that the optimal shell morphology for burrowing depended on the substrate the mussel was placed in. Less obese, thinner-shelled mussels were better burrowers in sand; the converse was true in mud. The pattern of optimal burrowing morphologies found in the laboratory experiment did not accurately predict morphological variation in the natural population. This could be because of i) a poor fitness surrogate (burrowing), ii) lack of important environmental variation in the experiment (e.g. turbulence), or iii) a non-adaptive pattern of morphological variation in the natural population.

In summary, variation in the morphology and growth rate of L. radiata is caused by phenotypic plasticity. The observed correlation between shell phenotype and habitat may represent differential adaptation, but an experiment where other fitness correlates are measured, and more realistic environmental variation is incorporated, is needed to test this hypothesis.

TABLE OF CONTENTS

	Page
CERTIFICATE OF EXAMINATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	viii
LIST OF TABLES	x
LIST OF FIGURES	xii
1. INTRODUCTION	1
1.1 Historical Hypotheses and Observations	2
1.2 Structure of the Thesis	9
2. MATERIALS AND METHODS	
2.1 Study Organism	11
2.2 Study Area	12
2.3 Variation in the Benthic Habitat	12
2.4 Correlation Between Habitat and Shell Form and Growth	16
2.5 Allozyme Variation	17
2.6 Heritability of Glochidia Size and Shape	19
2.7 Habitat Preference Experiment	22
2.8 Burrowing Experiment	27
3. RESULTS	
3.1 Variation in the Benthic Habitat	36
3.2 Correlation Between Habitat and Shell Form and Growth	36
3.3 Allozyme Variation	50

3.4 Heritability of Glochidia Size and Shape . . .	57
3.5 Habitat Preference Experiment	62
3.6 Burrowing Experiment	66
4. DISCUSSION	
4.1 The Exposure Gradient	72
4.2 Shell Morphology and Growth Rate of <u>L. radiata</u>	74
4.3 Allozyme Variation in <u>L. radiata</u>	80
4.4 Heritability of Glochidia Shell Morphology. .	84
4.5 Habitat Preference or "Stuck in the Mud"? . .	89
4.6 Shell Morphology as a Burrowing Adaptation. .	91
4.7 Conclusion - A Proposed Mechanism for the Observed Correlation	92
LITERATURE CITED	95
VITA	105

Correlations between species richness and exposure: Freshwater molluscs and macrophytes

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Abstract

I measured the correlation between a major environmental gradient in Inner Long Point Bay, Lake Erie, and the species richness of macrophyte and mollusc communities. This gradient of wind and current induced exposure in the benthic habitat was negatively correlated with macrophyte species richness ($r = -0.68$, $p < 0.001$), positively correlated with richness of the fingernail clam guild ($r = 0.58$, $p < 0.001$), and uncorrelated with the snail ($r = 0.53$) and filterfeeding mussel ($r = 0.18$, $p = 0.27$) guilds. Two models to explain the observed correlations are presented and compared. The "accelerated/retarded trajectory" model predicts that a disturbance such as exposure will either accelerate or retard the movement of a community to a general equilibrium, depending on the selectivity of the disturbance. The "gradient of equilibria" model postulates changes in the competition coefficients themselves with increasing disturbance, ultimately causing variation in species richness.

Introduction

Ecologists have long searched for some explanation of variation in species richness. Why do some habitats support numerous species of the same guild while others are relatively depauperate? It is commonly held that if a community is at "competitive equilibrium" (Huston, 1979) and there is no shortage of colonizing species, there will be as many species present as can be accommodated by the niche space (abundance and diversity of resources) available. However, predation or abiotic disturbance may act to keep population sizes below the "niche controlled" equilibrium densities and thereby reduce the intensity of competition in the community. Richness has often been observed to increase in response to such "intermediate disturbance" (e.g. Connell, 1970, 1978; Dayton, 1971; Menge, 1976; Paine, 1966, 1971,

1974; Keddy, 1983, 1984.

Yodzis (1978, 1985) argued that the effect of an intermediate level of disturbance on species richness depends on both the nature of the community and the selectivity of the disturbance. As an example, he cites both theoretical (Yodzis, 1977) and empirical (Addicott, 1974; Risch & Carroll, 1982) evidence for the hypothesis that species richness actually declines in niche controlled communities exposed to non-selective disturbance.

Niche controlled communities probably represent the traditional view of how communities are structured. But Yodzis (1978) also defined two kinds of communities (dominance and founder controlled) particularly relevant to benthic plants and animals. Species can co-exist in such systems without niche differentiation because of pure competition for space. Dominance controlled communities are those

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Begindatum werk	: 27-08-87
Ordernummer	: 750680
Naam cliënt	: NIJHOFF
Werktitel	: hydr-artikel
Artikelnnummer	: 7591
Vanaf kopijblad	: 001
Weggeschreven onder	: prof njih hydr-vw
Naam zelster	: Jellie de Vries

(in press, Hydrobiologia)

where the species compete for space and there is some, possibly cyclical hierarchy of ability to occupy and displace others from the space. Species in founder controlled communities also compete only for space, but the "winner" of a given piece of space is determined largely by which species occupies it first. This is primarily a function of the relative speed of colonization of the member species.

Yodzis (1978, 1985) predictions of the effects of disturbance on each of these community types basically reflect either an acceleration or a retardation of a community's movement to equilibrium. If disturbance selectively impacts sub-dominants, they are eliminated more quickly from the community and lower richness results. If disturbance primarily impacts the dominant species, elimination of sub-dominants is prevented or retarded and increased richness results. I will call this an "accelerated trajectory" or "retarded trajectory" model of varying richness, depending on the impact of the disturbance.

Another possible cause of variation in species richness along a disturbance gradient is covariability of the competition coefficients with the disturbance. A single dominant species in a habitat with little dis-

turbance may be a poor competitor in more disturbed areas. Its replacement by several species in habitats with significant disturbance may mimic the predictions of Yodzis' model, but the changes in richness would be the result of new equilibrium points along the disturbance axis rather than accelerated or retarded movement to a general equilibrium. I will call this a "gradient of equilibria" model.

In this study I measured the relationship between an environmental gradient of disturbance (wind and current induced turbulence or "exposure" in Inner Long Point Bay, Lake Erie) and the species richness of freshwater molluscs and aquatic macrophytes in the bay. I will show that both of the models described are consistent with the data, and I will propose experiments which will increase our understanding of how species richness varies in these communities.

Materials and methods

In June, 1984, 11 transects were sampled by boat in Inner Long Point Bay, Lake Erie (Fig. 1; 42°38'N; 80°24'W). Every kilometre along a transect the boat

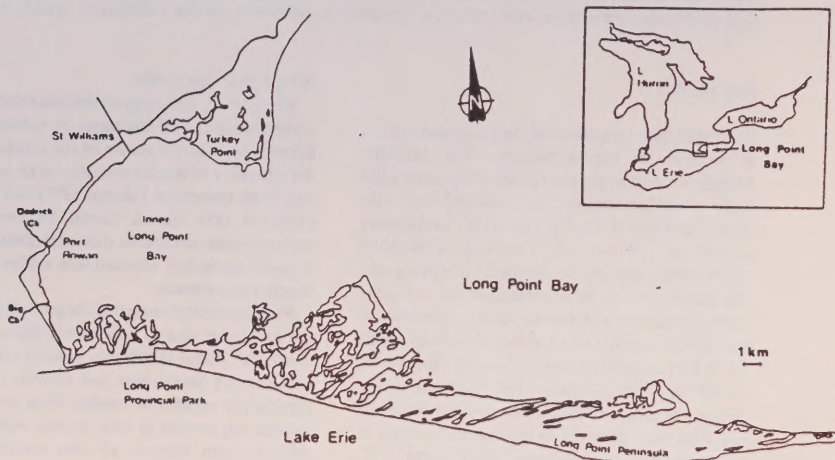


Fig. 1. The study area.

was anchored and a sampling site was established. The transects were chosen in an effort to equally allocate the sites to near (< 1 km) and offshore areas. A total of 41 sites were sampled.

At each site a plastic, circular sampling frame (i.d. = 1 m) was used to sample three randomly chosen subsites within 5 m of the boat. I used SCUBA at each of the subsites to measure depth, hand-collect all mussels in the frame area, collect or site-identify macrophytes, take two sediment samples for small molluscs and sediment analyses respectively, and collect a water sample in a Pyrex stoppered bottle. All samples were placed in a cooler immediately.

Within 12 h the mussels were cleaned and identified, one sediment sample from each subsite was sieved and hand-picked for small molluscs (which were preserved in 40% ethanol), reference macrophytes were placed in a plant press, and the water samples were processed. The pH was measured with a Fisher Accumet pH meter (Model 156). Alkalinity and calcium were determined titrimetrically as described in ASTM (1978).

Analyses of sediment samples from each subsite were done over a two month period, so the samples were kept frozen until analysis. Loss on ignition (LOI) of the sediments was estimated by drying a sample at 80°C, cooling, weighing out a small (< 25 g) subsample, baking at 550°C for one hour, cooling, and reweighing. LOI was the proportion of weight lost during the baking process. Sand content was the percentage of a dried, 250 g subsample of the sediment which did not pass through a 74 micron (No. 200) sieve after wet sieving. No sediments contained any particles larger than coarse sand.

Macrophyte identifications were made using Fasset (1975), Prescott (1962), and Gleason and Cronquist (1963). Mussels (Unionidae) were identified using Clarke (1981) and were verified by J. M. Topping (Nat. Mus. Canada). Clarke (1981) and Mackie *et al.* (1980) were used to identify the gastropods, and G. L. Mackie (University of Guelph) identified or verified the Pisidiidae.

Environmental and biological data for each site were obtained by pooling data from the three subsites. Thus, if a species occurred in only one of the three subsites at a particular site, it was considered

"present" at that site. For each environmental variable, the mean of the three subsites was used to represent the site.

The major environmental gradient in the bay was determined using principal component analysis (PCA; using SAS PROC PRINCOMP [SAS Institute 1982] on the covariance matrix of the log-transformed (except for pH) environmental data (alkalinity, calcium, pH, depth, % sand, LOI). These variables adequately reflect both the water and sediment quality at each site, and are temporally stable relative to other possible descriptors. Log transformations were used to improve the linearity of relationships among the variables. Pearson correlations and scatter plots were used to assess the strength and nature of the relationship between the score of a site on the major environmental gradient (i.e. the first principal component) and the species richness of the three molluscan guilds (grazing gastropods, filterfeeding mussels, deposit-feeding finngail clams). The relationship between the environmental gradient and macrophyte species richness was also examined by the same procedure.

Results

Substrate characteristics of Inner Long Point Bay were much more variable among the sites than either water chemistry or depth (Table 1). This is also indicated in the results of the PCA (Table 2; Fig. 2), which showed only one meaningful gradient in the environmental data: mucky (low % sand, high LOI) to sandy (high % sand, low LOI) sites. I will interpret this as a gradient of "exposure", or wind and current

Table 1. Means, ranges, and coefficients of variation of environmental variables (n = 41).

	MEAN	RANGE	C.V.
Alkalinity (meq L ⁻¹)	2.34	1.89 - 2.99	13
Calcium (mg L ⁻¹)	39.4	32.7 - 50.0	12
pH	8.4	8.2 - 9.0	2
Depth (m)	2.53	1.17 - 3.70	22
% Sand	50.0	2.4 - 99.2	68
% L.O.I.	2.5	0.3 - 6.3	70

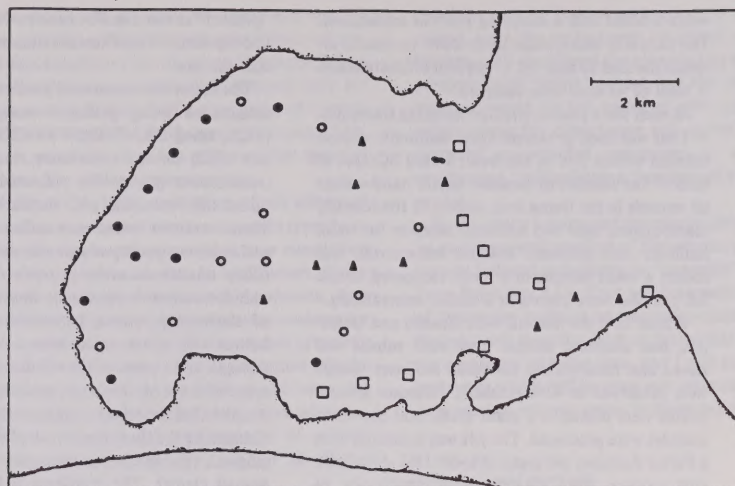


Fig. 2. Quartiles of sites on the exposure gradient as measured by their PCI scores ($\square$ = very high; $\triangle$ = high; $\circ$ = medium; $\bullet$ = low).

Table 2. Principal component analysis of the log-transformed (except pH) environmental data.

	Eigenvectors		
	PC1	PC2	PC3
Alkalinity	-0.08	-0.04	-0.20
Calcium	-0.07	-0.04	-0.06
pH	0.06	-0.12	-0.12
Depth	-0.06	0.16	0.95
% Sand	0.80	0.59	-0.07
L.O.I.	-0.58	0.78	-0.18
Eigenvalue	1.16	0.08	0.06
% Explained	87	6	5

induced turbulence in the benthic environment.

I found 14 species of macrophytes in the bay (Table 3). *Chara vulgaris* and *Vallisneria americana* were the two most common species. Three distinct guilds of molluscs were found (Table 4). There were nine species of snails (Gastropoda) present, with *Amnicola limosa* and *Pleurocera acuta* each occur-

Table 3. Macrophyte species and frequencies.

Species	Frequency (out of 41 sites)
<i>Anacharis canadensis</i> (Michx.) Rich.	8
<i>Ceratophyllum demersum</i> L.	5
<i>Chara vulgaris</i> L.	27
<i>Myriophyllum spicatum</i> L.	12
<i>Najas flexilis</i> (Willd.) Rostk. & Schmidt	13
<i>Nitella flexilis</i> (L.) C.A. Agardh	1
<i>Potamogeton illinoensis</i> Morong	1
<i>Potamogeton crispus</i> L.	1
<i>Potamogeton filiformis</i> Pers.	1
<i>Potamogeton gramineus</i> L.	1
<i>Potamogeton pectinatus</i> L.	1
<i>Potamogeton praelongus</i> Wulfer	10
<i>Vallisneria americana</i> Michx.	26

ing in over half of the sites. The most frequently occurring mussels (Unionidae) of the ten species found were *Lampsilis radiata* and *Anodonta grandis*. Of the 14 fingernail clam (Pisidiidae) species present, *Pisidium nitidum* was clearly the most common.

Table 4. Mollusc species and frequencies.

Species	Frequency (out of 41 sites)
GASTROPODA (snails)	
<i>Amnicola limosa</i> (Say, 1817)	24
<i>Campelema decisum</i> (Say, 1816)	3
<i>Helisoma trivolis</i> (Say, 1816)	1
<i>Marstonia decepta</i> (Baker, 1928)	1
<i>Physa gyrina</i> Say, 1821	7
<i>Pleurocera acuta</i> Rafinesque, 1831	24
<i>Pseudosuccinea columella</i> (Say, 1817)	1
<i>Valvata sincera</i> Say, 1824	6
<i>Valvata tricarinata</i> (Say, 1817)	7
UNIONIDAE (mussels)	
<i>Anodonta grandis</i> Say, 1829	13
<i>Anodonta imbecilis</i> Say, 1829	3
<i>Elliptio dilatata</i> (Rafinesque, 1820)	3
<i>Fusconia flava</i> (Rafinesque, 1820)	2
<i>Lampsilis radiata</i> (Gmelin, 1792)	23
<i>Lampsilis ventricosa</i> (Barnes, 1823)	2
<i>Ligumia nasuta</i> (Say, 1817)	10
<i>Pleurobema coccineum</i> (Conrad, 1836)	1
<i>Proptera alata</i> (Say, 1817)	3
<i>Villosa iris</i> (Lea, 1830)	1
PISIDIIDAE (fingernail clams)	
<i>Musculium lacustre</i> (Muller, 1774)	3
<i>Musculium partumeium</i> (Say, 1822)	11
<i>Musculium transversum</i> (Say, 1829)	9
<i>Pisidium casertanum</i> (Poli, 1795)	12
<i>Pisidium compressum</i> Prime, 1852	10
<i>Pisidium equilaterale</i> Prime, 1852	1
<i>Pisidium lilljeborgi</i> Clessen, 1886	3
<i>Pisidium nitidum</i> Jenyns, 1832	21
<i>Pisidium rotundatum</i> Prime, 1852	5
<i>Pisidium subtruncatum</i> Malm, 1855	3
<i>Pisidium variabile</i> Prime, 1852	5
<i>Pisidium ventricosum</i> Prime, 1851	1
<i>Shaerium corneum</i> (Linnaeus, 1758)	9
<i>Sphaerium striatinum</i> (Lamarck, 1818)	2

Although other macrobenthos (i.e. insects, crustaceans) were found, they were not identified. The molluscs were clearly dominant both numerically and with respect to biomass.

The species richness of the macrophytes and the three molluscan guilds are presented in Table 5, together with their correlations with the exposure gradient (as defined by the PCA). The macrophyte and fingernail clam assemblies have strong, opposite correlations with the exposure gradient (Fig. 3). The

Table 5. Means, ranges, and correlations with exposure of macrophyte and mollusc species richness ($n = 41$).

	MEAN	RANGE	EXPOSURE 'r'
Macrophytes	2.6	1–6	–0.68***
Snails (Gastropoda)	1.8	0–4	0.10 ^{NS}
Mussels (Unionidae)	1.5	0–4	0.18 ^{NS}
Fingernail Clams (Pisidiidae)	2.3	0–9	0.58***

(***: $p < 0.001$, NS: $p > 0.25$)

nature of these correlations was clarified by comparing the five most exposed sites, with a mean LOI of 0.6% (range of 0.29 to 0.69%) and a mean % Sand of 94.9% (82.5 – 99.2%), to the five least exposed sites, with a mean LOI of 4.3% (3.1 – 5.7%) and a mean % Sand of 9.2% (6.6 – 11.4%).

The five lowest exposure sites had a mean of 0.6 (0–1) fingernail clam species and a mean of 3.8 (3–5) macrophyte species. The only pisidiid species found at these sites were *M. partumeium*, *P. nitidum*, and *P. compressum*. Four of the five lowest exposure sites had *Anacharis canadensis*, *Najas flexilis*, *Valisneria americana*, and *Myriophyllum spicatum*.

The five highest exposure sites had a mean of 3.6 (2–8) pisidiid species and 1.8 (1–3) macrophyte species. *Chara* was the only macrophyte present in all five high exposure sites, and it occurred alone in two of these five sites. Of the fingernail calms, *P. casertanum* occurred in four of the five sites, but no other species occurred in more than two of the five sites.

Discussion

The exposure gradient

The fine sediments of Inner Long Point Bay originate as deltaic deposits of Big Creek and Dedrick Creek, the primary inflows (Heathcote, 1981), with some contribution from decomposing macrophyte and plankton debris. The position of a site on the "exposure" gradient defined in this study depends on the sources, transport, re-suspension, and accumulation of this fine material. This is determined by the location of the primary inflows (the southwest

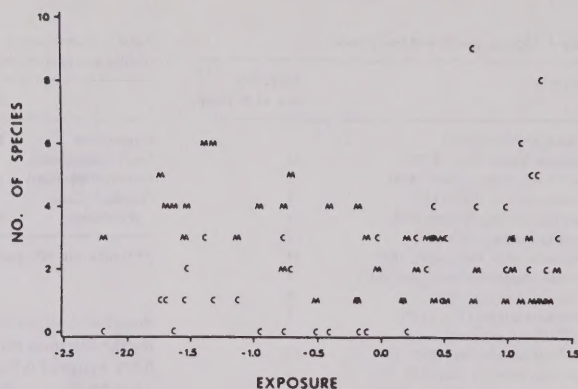


Fig. 3. Species richness of macrophytes (M) and fingernail clams (C) in relation to exposure (the score of each site on the first principal component of the environmental data).

corner of the bay; Fig. 1) and the prevailing winds, which are from the southwest (Kohli & Farooqui, 1980). Wind-induced shallow waves (Wetzel, 1975) will cause increasing benthic turbulence as one moves away from the southwest shoreline. These winds also generate surface seiches, with amplitudes as high as 2 m in Lake Erie (Wetzel, 1975), which will "flush" the part of the Inner Bay not protected by Turkey Point (the northeastern gate of the bay) on a southwest/northeast axis. Considered together, these factors explain the distribution of high, medium, and low exposure sites in Inner Long Point Bay (Fig. 2).

The macrophyte community

Chara vulgaris appears to be functionally dominant at sites with high exposure, perhaps because it is better able to maintain attachment in more turbulent areas than other species. It is also much less reliant on nutrient uptake through roots than the angiosperms, so less organic sediments do not hinder its growth. Wilson and Keddy (1986a) argued that macrophytes with small rosettes are more resistant to wave damage than those with tall, leafy shoots,

and thus are better competitors at high exposure sites. *Chara*, with its low-lying stalks and finely divided "leaves" also appears to be morphologically adapted to more turbulent habitats. In less exposed sites it may suffer from shading by the taller, broader-leaved species.

Thus, the correlation between species richness and exposure found in Inner Long Point Bay may have been caused by a "flip-flop" in competitive superiority as exposure increased (the "gradient of equilibria" model). When measured in an undisturbed, organic-rich habitat, macrophyte species from low exposure areas had greater competitive ability than those from high exposure sites (Wilson & Keddy, 1986a). But relative competitive ability may vary substantially if measured at different points along the disturbance gradient. Wilson and Keddy's results may have been different had their experiment been repeated in a more disturbed habitat.

Alternatively, the leafy macrophytes may be considered functional dominants that are selectively impacted by increasing wave exposure. Such a disturbance should enable sub-dominants to maintain a presence and species richness to increase (Yodanis, 1985). Wilson & Keddy (1986b) observed a decline in diffuse competition among macrophyte species

with increasing exposure, which would support such a "retarded trajectory" model. The reduction in richness observed in the present study may just represent "excessive" rather "intermediate" disturbance, and the inability of species other than *Chara* to resist local extinction at high exposure. Peaks in species richness at an intermediate level of exposure have been observed in shoreline macrophyte communities (Keddy, 1983, 1984).

The fingernail clam community

There is evidence that both niche differentiation and dominance hierarchies help to structure fingernail clam communities. *Sphaerium* and *Musculium* species are epifaunal, while *Pisidium* spp. are infaunal, so these two groups may coexist in a given patch by niche differentiation. A field experiment has shown that inter-specific competition between congeneric species of the Pisidiidae occurs, and may be more important than intra-specific competition (Mackie *et al.*, 1978; Connell, 1983).

Mackie *et al.* (1978) found *Musculium transversum* to be competitively superior to *M. securis* in shallow water, and predicted that *M. transversum* would eventually eliminate *M. securis* in similar habitats if they occurred together. In deeper waters they found no evidence of inter-specific competition. In this study *M. transversum* was numerically dominant in areas of low exposure. If it is functionally dominant in these areas, perhaps its numbers are sufficiently reduced at high exposure for other species to coexist. Either physical disturbance or increased predation by waterfowl in the exposed areas (Smith, 1979) may cause such a reduction in *M. transversum*. Richness would be higher in the more exposed areas, as predicted by the "retarded trajectory" model.

Alternatively the exposure gradient may be correlated with changes in the dominance hierarchy, effectively removing *M. transversum* as a top competitor at high exposure, increasing the proportion of contingent competitive interactions (Yodzis, 1985), and thereby increasing richness.

Non-significant correlations: Snails and mussels

The species richness of two molluscan guilds (the snails and the mussels) showed no relationship with the exposure gradient. This was unexpected for the snails because plant richness was highly correlated with the gradient and macrophytes are an important component of the feeding and habitat niche of the gastropods. MacArthur & MacArthur (1961) found with increasing structural diversity of the vegetation, the diversity of birds using the vegetation increased. The lack of such a relationship in my data may indicate i) lack of real structural diversity in the macrophyte species or ii) broad niches with respect to macrophyte preference in the snails. I would tentatively reject the first hypothesis because of the diverse morphology of the macrophytes found. The second explanation seems more likely, in which case the exposure gradient is likely having little general effect on the life habits of the gastropod guild. The mussels (Unionidae) appear to have adequate within-species plasticity (e.g. Hinch *et al.*, 1986) to be similarly well-buffered from the exposure gradient, at least at the community level.

Choosing a model: Beyond correlational data

Both the "retarded trajectory" and the "gradient of equilibria" models have been used to explain the observed correlations between species richness and exposure. The predominance of one or the other in explaining field observations depends on one's knowledge of the competitive interactions in a community at various points along a disturbance gradient. In particular, the relationship between diffuse competition and the level of disturbance must be evaluated experimentally (e.g. Wilson & Keddy, 1986b). If competition is important at intermediate levels of disturbance, changes in the dominance hierarchy with variation in disturbance must be measured. If it is relatively unimportant, then the selectivity of the disturbance and the availability of hardy species will determine the effect of the disturbance on species richness, as predicted in the "accelerated/retarded trajectory" model. Competition may even be of little importance when disturbance (as de-

fined here) is minimal. Thus, although the data are consistent with both of the "competition-based" models discussed, neither may be an appropriate description of the system. The available evidence suggests that for macrophytes, diffuse competition is important at low levels of shoreline exposure, but reduced at higher levels (Wilson & Keddy, 1986b), supporting the "retarded trajectory" model. Although the same may be true for the finernail clam community, clear evidence is lacking.

Summary

Two communities in Inner Long Point Bay, Lake Erie showed strong, opposite correlations between species richness and exposure or turbulence in the benthic habitat. Macrophyte richness was negatively correlated ($r = -0.68$, $p < 0.001$) with exposure, while fingernail clam richness was positively correlated ($r = 0.58$, $p < 0.001$) with the same gradient. Two models, one which predicts changes in richness as a result of a disturbance-caused decline in the importance of competition, and one which predicts the same changes as a result of a disturbance-caused change in competition coefficients, are used to explain the data. Tests for determining which (if indeed either) of these models is appropriate are proposed.

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Within-basin Variation in the Shell Morphology
and Growth Rate of a Freshwater Mussel

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ABSTRACT

We measured a correlation between the habitat of the freshwater mussel Lampsilis radiata siliquoidea (Barnes 1823) from several sites in Inner Long Point Bay, Lake Erie, and its shell morphology and growth rate. Morphometric analysis revealed that mussels in more exposed areas of the bay (areas with more turbulence and sandier sediments) had thicker shells than those from less exposed areas (areas with little turbulence and muddier sediments). Two growth rate analyses showed that the mussels from high exposure areas were also faster growing. The results demonstrate the importance of i) a multivariate consideration of shell form (as opposed to the use of ratio variables) when assessing form-habitat correlations, ii) quantitatively defining habitat variation in examining such relationships, and iii) measuring form-habitat relationships among more than two sites in a given basin or stream reach.

INTRODUCTION

There is a long history (reviewed in Ball 1922; Eagar 1948; Tevesz and Carter 1980) of observed correlations between the shell characteristics (i.e. form and growth rate) of unionacean bivalves and their habitat. Three relationships concerning exposure in the benthic habitat (shear stress in streams, benthic turbulence in lakes; see Bailey 1987) have been observed in many unionid species :

- i) Shell size and thickness (i.e. growth rate) increase with increasing exposure.
- ii) Obesity (W/L; Fig. 1) and relative height (H/L; Fig.1) of the shell decrease with increasing exposure.
- iii) Shells with straight ventral and arched dorsal margins (Fig. 1) are found in areas of high exposure.

Such observations usually have been only "semi-quantitative", with poorly defined habitat descriptors and frequent use of ratio variables to describe morphometric variation. A poorly defined habitat limits resolution in measuring the strength and nature of a relationship between habitat and shell form. The use of ratio variables may lead to misinterpretation of the trends present, as well as a significant loss of information concerning the variation in shell morphology (Green 1986). Explicit consideration of allometric relationships among different shell dimensions (i.e. multivariate allometry; Jolicoeur 1963) is preferable.

Studies of freshwater mussels using more suitable morphometric and growth-rate analyses have been restricted to two collecting sites only of different exposures in a given basin (Hinch et al. 1986; Hinch and Bailey 1988). Mitchell and Collins (1984) measured growth rates of Elliptio complanata at several sites in each of 10 southeastern Ontario

lakes. They concluded that substantial variability in shell growth rates can occur within a basin. It may therefore be insufficient to examine shell differences between mussels at two sites only in a given basin. In this study we examined variation in the shell form and growth rate of Lampsilis radiata siliquoidea (hereafter "L. radiata") collected from several sites in each of three areas of varying exposure in Inner Long Point Bay, Lake Erie (hereafter "IB").

MATERIALS AND METHODS

In September, 1984, L. radiata were collected in each of three areas of IB (Fig. 2). Based on previously collected environmental data (Bailey 1988), these areas spanned a range of high, medium, and low exposure. This "exposure gradient" in IB was defined by a principal component analysis of the covariation of several environmental variables (pH, alkalinity, calcium, depth, per cent sand in the sediment and per cent loss on ignition [LOI] of the sediment) at 41 sites in Inner Long Point Bay (see Bailey 1988). The major environmental gradient (first principal component) showed sites ranging from "mucky" (low per cent sand; high per cent LOI) to "sandy" (high per cent sand; low per cent LOI). This was interpreted by Bailey (1988) as a gradient of low to high "exposure" or turbulence in the benthic habitat.

To verify that a range of exposure levels was sampled in this study, we collected sediment samples from each of the areas and "scored" the sites on the exposure axis defined in Bailey (1988). Several "very high" exposure sites were visited, but there were no L. radiata present. The mussels were hand-collected using SCUBA searches which were based at three collecting sites within each of the three areas (Fig. 2). Morphometric data from mussels collected at the three collecting sites within each exposure area were pooled. This allowed variability among the collecting sites within an exposure area to be weighted by the number of mussels collected from each site. This could be deemed "sacrificial pseudoreplication" (Hurlbert 1984), so results of statistical tests reported must be considered liberal. The nature of differences between exposure areas, however, is more accurately assessed than it would be with a hierarchical statistical design.

We collected and measured 193 male L. radiata (73 high; 77 medium;

43 low exposure). The females collected were excluded from further analyses because L. radiata is sexually dimorphic with respect to shell form (Clarke 1981), and there were too few females in the sample to properly characterise their morphology and growth pattern.

The shells were cleaned, air dried, and weighed (in grams) with a Mettler PC4400 electronic balance. The length, height, and width (Fig. 1) of each shell were then measured (in mm's) with Mitutoyo 500-115 Digimatic Calipers. Canonical variates analysis (CVA; Reyment et al. 1984), as implemented in SAS PROC CANDISC (SAS Institute Inc. 1985), was used to compare the shell morphology of mussels from the three exposure areas. Scatter plots showed allometric relationships among the variables, so (natural) logarithmic transformations of all variables were used in the CVA.

To compare growth rates among the three exposure groups, we did a Walford plot analysis of consecutive annual ring measurements, as in McCuaig and Green (1983). Then we used analysis of covariance (ANCOVA) to compare the Walford plot regression lines for mussels from each exposure level. Also, as an independent comparison of growth in the first year of life, the length of the first annual ring was measured and compared among the three groups using one-way ANOVA.

RESULTS

Variation in Shell Morphology

CVA showed that there was a highly significant difference in shell morphology among L. radiata from the three exposure areas (Table 1). Only the first canonical axis was important in describing differences among the groups. The mean canonical scores for mussels from each of the three exposure levels, together with the standardised and structure coefficients (Table 1), indicated that as exposure increased, shell thickness (i.e. weight/length ratio) increased. This is illustrated with a 95% confidence ellipse of the mean weight and length for each group (Fig. 3). All three groups had about the same (geometric) mean length (59mm), but the average shell weights at this length were 21.1g (high exposure), 18.5g (medium exposure), and 17.1g (low exposure).

Variation in Shell Growth Rate

ANCOVA of consecutive ring measurements (the Walford plot technique) showed that the estimated time to reach asymptotic size was the same in the three groups (i.e. the slopes of the Walford plots were the same), so I compared the absolute growth rates (i.e. the intercepts of the Walford plots). There were significant differences ($p < 0.001$) among the three intercepts (Tables 2,3), and a posteriori contrasts indicated that mussels from the high exposure area had significantly ($p < 0.01$) faster growth rates (i.e. larger Walford intercepts) than those from medium or low exposure areas. Growth rates of mussels from the low and medium exposures were statistically indistinguishable ($p > 0.5$). The von Bertalanffy growth curves derived from the Walford plot analysis (as described in McCuaig and Green 1983) indicate the faster growth of the high exposure mussels (Table 3; Fig. 4).

In another independent comparison of growth rates, length of the

first annual ring also differed significantly among the three groups ($F=13.7$; $df=2,150$; $p<0.001$). Again, growth was faster in mussels from relatively high exposure areas (means : high=13.6mm; medium=12.6mm; low=11.2mm).

DISCUSSION

Variation in Shell Morphology

Ortmann (1920), in compiling evidence for what later became known as "Ortmann's Law" (mussels from downstream depositional areas in a stream are more obese than those from upstream erosional areas), noted that many unionid species (including L. radiata) do not show this correlation. He hypothesized that the relationship would only be observed in mussels from the more primitive taxonomic groups. Although he did not define "primitive" in this context, Ortmann (1920) mentioned that the genera Fusconaia, Amblema, Quadrula, and Pleurobema "belong to the most primitive types of North American Naiades".

Our results for L. radiata agreed with those of Ortmann (1920), in that we found no relationship between shell obesity and exposure. However, another relationship (increased shell thickness and growth rate with greater exposure) was found. A correlation between exposure and shell morphology may exist in many species previously thought to be "unresponsive" to variation in exposure. It could be obscured by only looking at ratios of morphological measurements, which may distort or not show the relationship of interest (Atchley et al. 1976; Atchley 1978; Atchley and Anderson 1978). The precise nature of the relationship between exposure and shell morphology will vary among species depending on allometric relationships among the various morphometric variables. This would be true even if the effect of exposure was identical for each species (e.g. increased growth rate). It is clear that a multivariate consideration of shell morphology (e.g. Green 1972; Hinch et al. 1986; Hinch and Bailey 1988) is necessary to properly characterise the form of the shell.

Hinch et al. (1986) observed differences in shell morphology and

growth rate between L. radiata collected from a sand (i.e. high exposure) and a mud (i.e. low exposure) site in IB. They then used a transplant experiment to separate short and long term (possibly genetic) interactions between the habitat and growth in various dimensions of the mussel. Although Hinch et al.'s (1986) growth rate comparison gave the same result as ours (see below), they found smaller, more obese shells in the mud habitat. We found no evidence of differences in obesity with variation in exposure. Since their comparison of high and low exposure areas is based on only one collecting site in each area, the relationship observed in our study better describes the general situation in IB.

Variation in the shell morphology of freshwater mussels in relation to exposure has been explained both as an adaptive and non-adaptive pattern. More "robust" shells (i.e. thicker, larger shells) may help a mussel maintain its position in turbulent habitats, while smaller, thinner shells may prevent sinking in softer substrates (Wilson and Clark 1914; Hinch et al. 1986). Such an explanation requires either adaptive plasticity (Eagar 1978) or genetic divergence (Ortmann 1920; Kat 1984) with respect to shell form. Alternatively, the correlation between shell form and habitat may reflect a non-adaptive response to variation in feeding efficiency (Kat 1982) or food supply (Stansbery 1970).

Brown et al. (1938) reported finding "stunted" L. radiata in highly exposed lentic habitats. They sampled L. radiata in an extremely exposed area of western Lake Erie off Pelee Island. Compared to specimens collected in less exposed areas near to this site, the mussels were very small. Green (1972) also concluded that L. radiata in very exposed

habitats showed reduced growth rates. The results of Brown et al. (1938) and Green (1972), combined with ours and those of Hinch et al. (1986), suggest that the positive relationship between exposure and growth rate, whether representative of an adaptive or non-adaptive pattern, has a limited range beyond which L. radiata is unable to function and grow efficiently (Hinch and Bailey 1988). We tried to test this hypothesis by sampling L. radiata in Outer Long Point Bay along the northern shore of Long Point, but in the highly exposed, sandy, "washboard bottom" habitats found in this area, there were no mussels present. Brown et al. (1938) noted that at the highly exposed Pelee Island site mussels were cast up along the shore during days of only mild westerly winds, and many dead mussels (with bodies still intact) were found in the shore drift.

Variation in Shell Growth Rate

The positive relationship between growth rate and exposure found in this study was similar to that found by Hinch et al. (1986) for L. radiata from a sandy and a muddy site in IB. The small differences between Hinch et al.'s (1986) and our estimates of the coefficients for the Walford plot regression lines are probably due to sampling error, to differences in the range of ring measurements used to compute the regression lines (Hinch et al. [1986] generally used larger pairs of rings), and the different set of sites used in each study.

Shells from mussels living in high exposure habitats were thicker but not longer than those from lower exposure areas (according to the CVA of shell morphology), and yet both of the growth rate analyses (Walford plot; length of first annual ring) showed faster growth in length in the high exposure mussels. This implies one of two things : either the growth curve of each exposure group changes as age increases

beyond the range of annual ring data collected, or there is a higher mortality rate in the high exposure mussels. The first scenario suggests the possibility of different growth strategies at different exposure levels : quick, early growth in the high exposure areas followed by a virtual cessation of growth in length; slow steady growth in the low exposure areas which continues beyond the age at which high exposure mussels have stopped growing. This seems unlikely, since a reciprocal transplant experiment over one growing season (Hinch et al. 1986) showed faster growth in high exposure mussels even though all animals used in the experiment were close to asymptotic size. The second scenario, higher mortality in mussels living in high exposure areas, also suggests the possibility of different strategies for reproduction and growth (maintained either through adaptive plasticity or genetic divergence and reproductive isolation), but no data are available to adequately test such an idea.

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Table 1. Canonical variates analysis of variation in shell morphology among mussels from high, medium, and low exposure areas in IB. Standardised coefficients are eigenvectors multiplied by the "pooled within" standard deviation of each variable. Structure coefficients are simple correlations between the CV scores and the original (log-transformed) variables.

	CV1		CV2	
	Standardised	Structure	Standardised	Structure
Weight	2.40	0.46	-0.69	0.27
Length	-1.92	-0.04	-0.29	0.40
Height	0.26	0.20	1.75	0.77
Width	-0.49	0.19	-0.24	0.20
Eigenvalue	0.34		0.004	
Canonical correlation	0.50		0.06	
Significance	F=7.5, df=8,374 p<0.0001		F=0.2, df=3,188 p=0.87	
Class means				
High	0.67		0.03	
Medium	-0.16		-0.07	
Low	-0.84		0.07	

Table 2. Analysis of covariance of consecutive growth rings using the Walford plot method.

Dependent variable = Length of 2nd ring (L2).

Covariate = Length of 1st ring (L1).

Grouping variable = Exposure (high, medium, or low).

Source	df	SS	MS	F	p
L1	1	10177.2	10177.2	1315.5	<0.0001
Exposure	2	323.2	161.6	20.9	<0.0001
L1 * Exposure	2	14.5	7.2	0.9	0.39
Error	152	1175.0	7.7		

Table 3. Walford plot regression lines and the derived von Bertalanffy growth curve equations for mussels from high, medium, and low exposure areas of IB.

Exposure	Walford Regression Line	von Bertalanffy Equation
High	$L_2 = 11.42 + 0.92 L_1$	$L_t = 142.8 (1 - e^{-.08t})$
Medium	$L_2 = 8.18 + 0.92 L_1$	$L_t = 102.2 (1 - e^{-.08t})$
Low	$L_2 = 8.65 + 0.92 L_1$	$L_t = 108.1 (1 - e^{-.08t})$

FIGURE CAPTIONS

Figure 1. Dimensions and orientation of a mussel (L=length, H=height, W=width, A=anterior, P=posterior, D=dorsal, V=ventral).

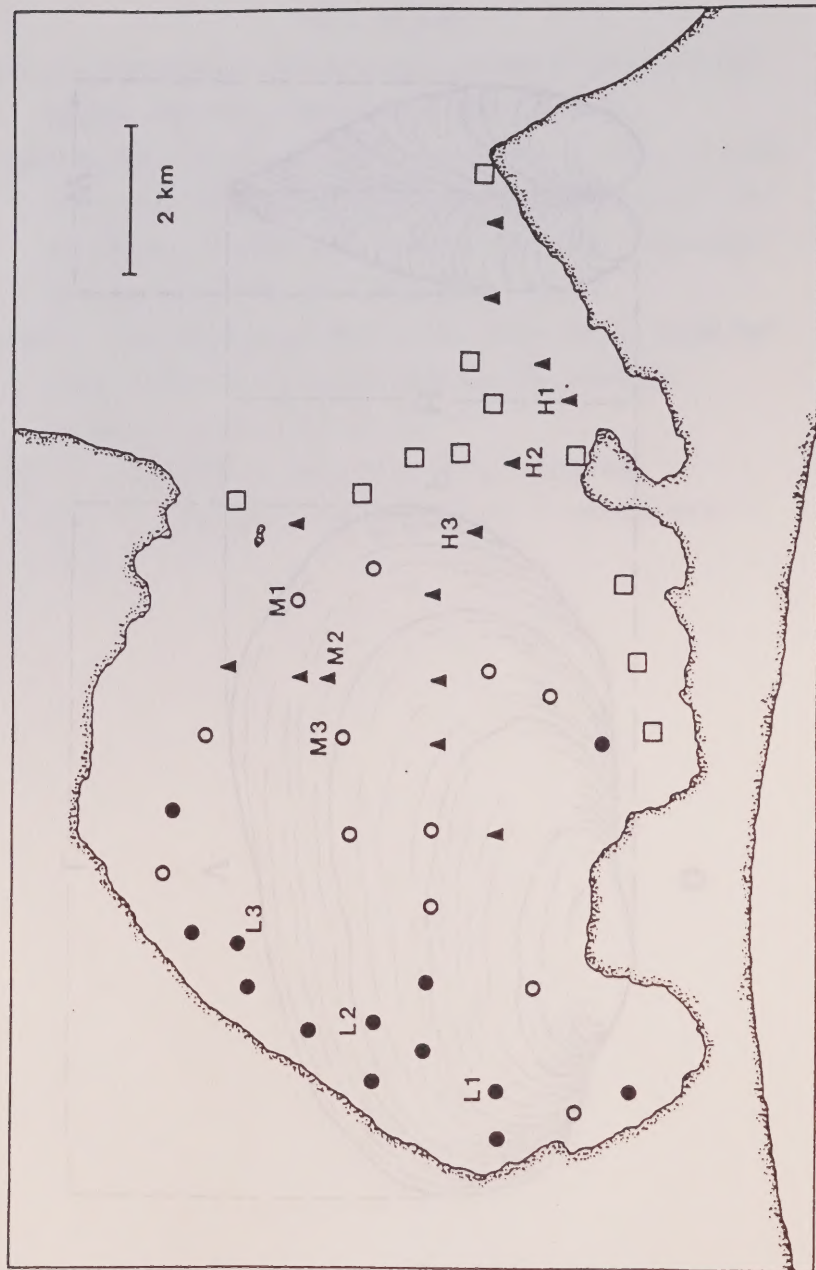
Figure 2. Quartiles for an exposure score ($\square$ =very high; $\blacktriangle$ =high; $\circ$ =medium; $\bullet$ =low), and mussel collecting sites (L1,L2,L3 = low area; M1,M2,M3 = medium area; H1,H2,H3 = high area). The exposure score was derived from survey data (see text).

Figure 3. Confidence ellipses (95%) for mean weight (g) and length (mm) (log-transformed) of L. radiata from high (H), medium (M), and low (L) exposure areas of IB.

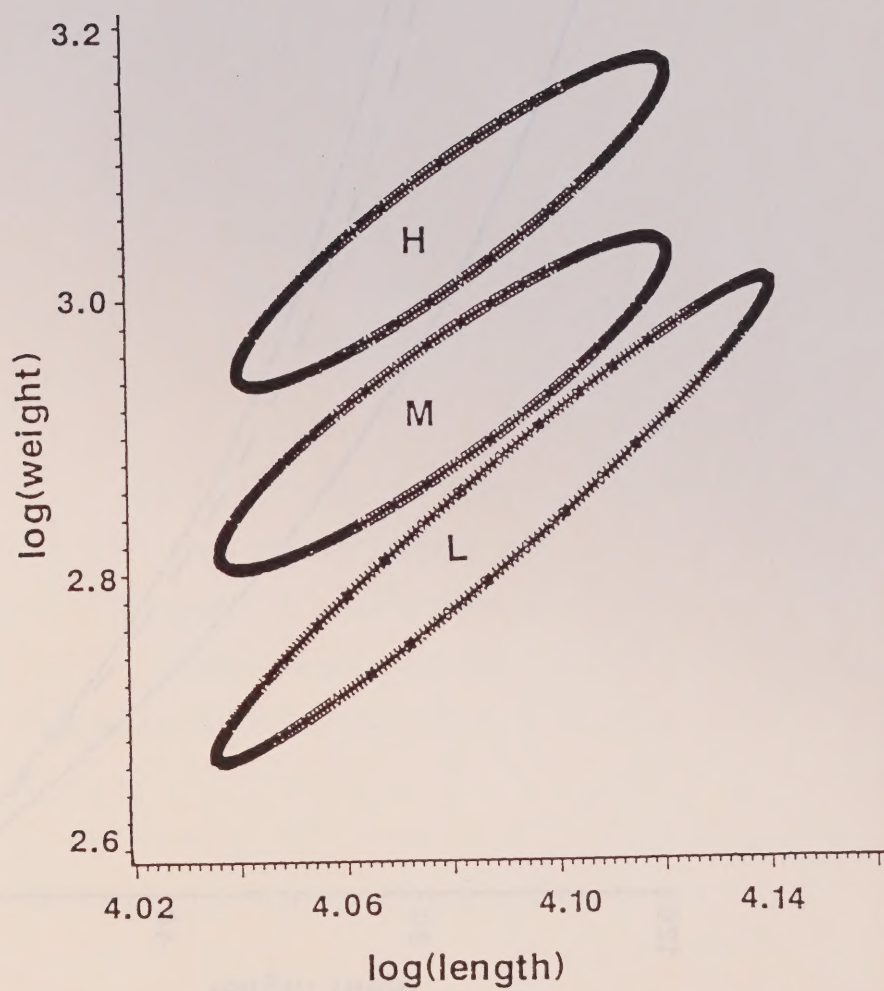
Figure 4. von Bertalanffy growth curves for L. radiata from high (—), medium (---), and low (--) exposure areas in IB.



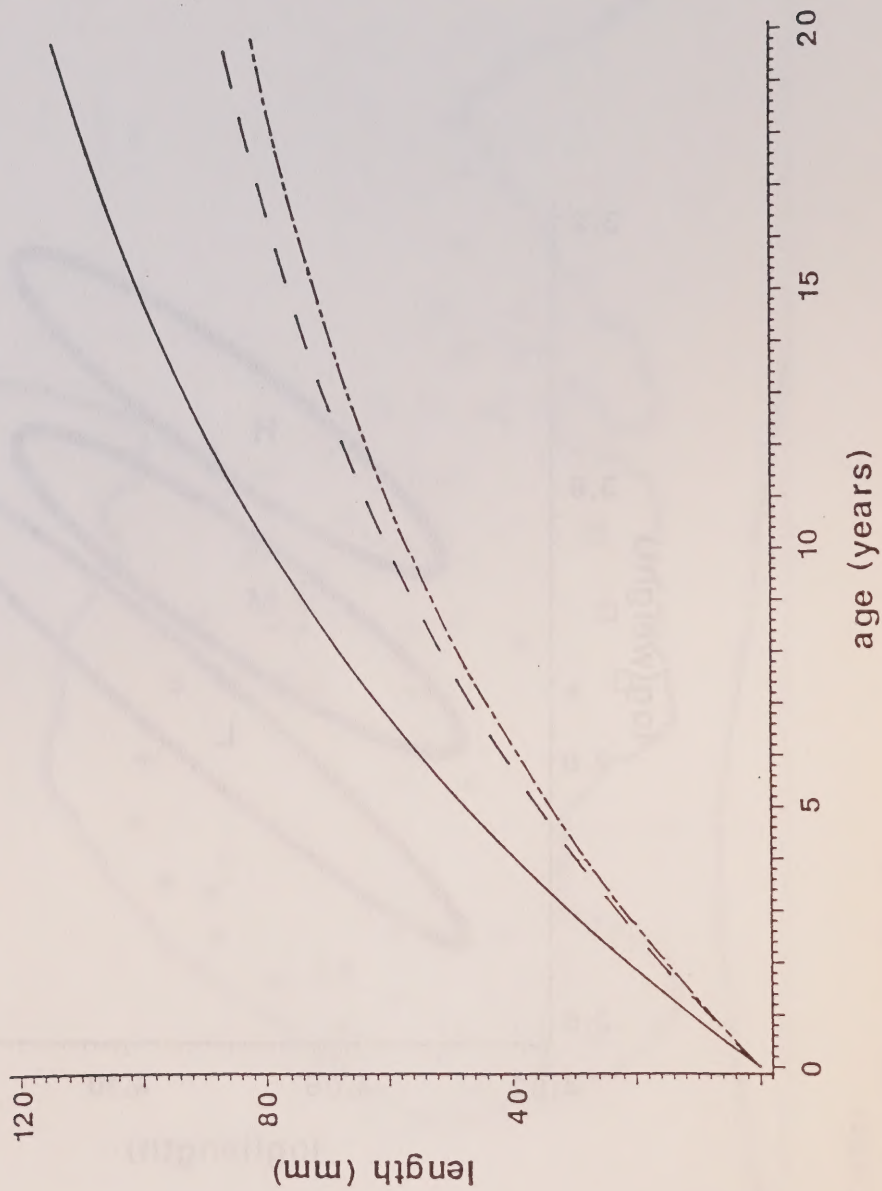
G.4231
Bailey / Green
Fig. 1



G.4231
Bailey / Green Fig. 2



G.4231
Bailey / Green Fig. 3



G. 4231
Railway Green Fig. 4

Temporal Changes in a Freshwater Mussel Population :

A Natural Experiment

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ABSTRACT

Bailey, R.C., Green, R.H., and Bailey, C. 1988. Temporal changes in a freshwater mussel population : a natural experiment. Can.J.Fish.Aq.Sci.

A population of Anodonta grandis in a small arctic lake previously sampled in 1973 was resampled in 1986. In both of the years sampled there about 14 times more mussels in deep (>1.5m) water than in shallow water, which supports the hypothesis that A. grandis evolved in and prefers deep-water habitats. There was a decline in growth rate, a shift towards an older age structure, and a drop in population density from 2.3 to 0.65 animals per m² between 1973 and 1986. This is consistent with the hypothesis that hydrocarbon pollution, obvious in this lake in 1973, has impacted this mussel population.

INTRODUCTION

In 1973 Green (1980) estimated the size distribution and density of a population of Anodonta grandis (Bivalvia; Unionidae) in Shell Lake, Inuvik, N.W.T. Out of over 1000 individuals sampled, he found no mussels less than 57mm in length (about 5 years old). Green proposed three possible explanations for this result:

i) Collecting bias : Young unionids are difficult to collect using sampling techniques more suitable for large individuals. Green (1980) sampled with SCUBA, using his bare hands to detect the mussels in the sediment.

ii) Naturally bad years : The years immediately prior to Green's sampling may have been poor for mussel recruitment due to variability in abiotic conditions or fish populations. Unionids rely on fish as hosts during a parasitic stage of their larval development.

iii) Hydrocarbon pollution : Shell Lake has served as an important float plane base since 1968 (i.e. five years before Green's sampling). By 1973 there were six private bases on this small (84.3 ha; $2060 \times 10^3 \text{ m}^3$) lake, and it continues today as a busy float plane "runway". The resulting hydrocarbon fuel pollution, which was "obvious" to both Green (1980) and Chang (1975) in 1973 could have seriously reduced recruitment in the mussel population (Green 1980).

To choose between these three hypotheses, the A. grandis population of Shell Lake was resampled in 1986. We predicted the following results, depending on which of the hypotheses was true :

i) Collecting bias : We would find the same lack of young mussels as Green (1980), but older individuals (up to about 100mm) would be well represented.

ii) Naturally bad years : We would find mussels born between 1972

and 1985, unless all of these years were "naturally bad", which seems highly unlikely. Thus, we should have found small individuals in our samples.

iii) Hydrocarbon pollution : If recruitment was severely reduced in 1968, there should be a low density of mussels in Shell Lake relative to the numbers obtained by Green (1980).

MATERIALS AND METHODS

Sampling was done by hand (using SCUBA) in late July, 1986, along the same four transects as used by Green (1980). Mussels were collected and measured as described in Green (1980). In addition, we kept 48 mussels from the large collection and measured two consecutive growth rings on their shells. This enabled us to estimate a growth curve for the population using the Walford plot technique, as described in McCuaig and Green (1984). We fitted the equation ' $L_{t+1} = a + bL_t$ ' using the geometric mean slope because of the error in measuring both L_2 and L_1 (McArdle 1988). We then generated the von Bertalanffy growth curve, ' $L_t = L_{\max} (1 - e^{kt})$ ', by recalling that ' $k = -\ln(b)$ ' and ' $L_{\max} = a/(1-b)$ '.

We used a loglinear model (Fienberg 1980) to examine correlations among the size class of the mussels (<60mm; 60 to 74mm; 75 to 89mm; >90mm), the depth of sampling (<2m; >2m) and the year of sampling (1973; 1986). SAS PROC CATMOD (SAS Institute 1985) was used to carry out the calculations.

A factorial ANOVA was used to assess the effect of depth (<1.5m; >1.5m) and year (1973; 1986) on the number of mussels collected from a pair of circular sample plots at a given site (total area = 4m^2). Different definitions of "deep" and "shallow" sites were used in the loglinear and ANOVA analyses in an effort to balance the number of observations in each cell for the analyses (Tables 1, 2). A regression of $\log(\text{variance})$ on $\log(\text{mean})$ for twenty paired samples indicated that something between a log and a square root transformation of the density data was appropriate prior to using parametric ANOVA (see Green 1979; pp.46-47). Statistical results using these two transformations did not differ, so we used $\log_e(x+1)$ transformations of the data (there

were some zero values) in the analysis reported here. SAS PROC GLM (SAS Institute 1985) was used to carry out the analysis.

RESULTS

Growth Rate

The Walford plot analysis of consecutive growth rings on 48 mussels resulted in the von Bertalanffy equation :

$$L_t = 145 (1 - e^{-0.06t}),$$

where ' L_t ' is length at time ' t '. In fitting the Walford model the simple linear model was sufficient ; there was no significant additional variance in L_{t+1} explained by L_t^2 (as in Green 1980). The growth curves for 1986 and 1973 are contrasted in Figure 1. These show slower growth in the 1986 population.

Length-Frequency Distribution

There was a significant three-way interaction among length-class, depth, and year ($\chi^2 = 14.78$; $df=3$; $p<0.003$), indicating that the difference between the length-frequency distribution of mussel shells in deep vs. shallow water changed between 1973 and 1986 (Table 2). In 1973 there was a greater proportion of large mussels present in deep water than in shallow. This was not the case in 1986, when length-frequency distributions were almost identical in deep and shallow water. Although there was a larger proportion of small mussels at both depths in the 1986 sample (i.e. about 8% in 1986, 1% in 1973), there were still fewer present than one would expect if we had sampled a population with a more or less stable age distribution using an unbiased technique. Looking at the largest three size classes, which were presumably sampled with less bias, there is a shift towards larger mussels in 1986 at both depths. The growth curves for the two years (Fig. 1) support the hypothesis of an older (rather than a faster-growing) population in 1986 relative to 1973.

Density

Factorial ANOVA of $\log(\text{density}+1)$ indicated no interaction between year and depth ($F < 0.01$; $df = 1, 44$; $p > 0.95$). There were independent effects of both year ($F = 4.49$; $df = 1, 44$; $p = 0.04$) and depth ($F = 16.95$; $df = 1, 44$; $p < 0.001$) on density. The geometric mean densities (per 4m^2) across both deep and shallow areas were 9.2 (95% c.i.: 4.2, 19) for 1973 and 2.7 (0.75, 6.7) for 1986. The confidence limits are asymmetrical because they have been back-transformed from logarithms. Geometric mean densities across both years were 16.3 (8.2, 31.4) for deep water and 1.2 (0.3, 3.7) for shallow water. These means have been calculated using the LSMEANS option in SAS PROC GLM (SAS Institute 1985), which corrects for unequal numbers of observations in the cells.

DISCUSSION

Depth Distribution of Anodonta grandis

In a small Ontario lake, Ghent et al. (1978) found greater numbers of A. grandis in deep water relative to the depth distribution of Elliptio complanata. They reported finding A. grandis to depths of 14m, and based on this and its "floater"-shaped shell speculated that it evolved primarily as a deep-water species. Our results agree with those of Ghent et al. (1978) ; we found greater numbers of mussels in deep water in both 1973 and 1986. Although Green (1980) fit a somewhat more complicated model to the 1973 density data, which included a quadratic relationship between $\log(\text{density}+1)$ and $\log(\text{depth})$, he too found that maximum predicted densities were at a depth of over 2m. A. grandis may be found at greater depths than other unionids as a result of i) a niche shift following competition with other species or ii) greater predation by Ondatra zibethicus (muskrat) in shallow water. There was very obvious evidence of muskrat predation at Shell Lake; muskrat middens (piles of empty shells) were very frequently observed. Headlee (1906) reported that muskrat predation limits the range of Anodonta spp. in shallow waters.

Impact of Disturbance on A. grandis

In 1986 we found fewer than one third the number of mussels that were present in 1973. We also found a shift in the size distribution of the mussels in the size classes least prone to biased sampling (i.e. the largest size classes). This shift, towards greater proportional representation by the largest size classes, indicated that the 1986 population was either older or faster-growing than the 1973 group. Comparison of growth curves generated using the Walford plot technique of analysing consecutive growth rings showed that growth was, if

anything, slower in the 1986 population. In summary, the combined density, growth, and length-frequency data support an hypothesis of a shrinking, aging population.

In his review of "Adverse Anthropogenic Effects on Mussels", Fuller (1974) cites evidence from Wilson and Clark (1912) and Baker (1928) that "gas works wastes, especially tar and oily scum" are "very damaging to mussels". If hydrocarbon pollution has caused the changes observed from 1973 to 1986 in the A. grandis population of Shell Lake, it must either have affected the young mussels directly or somehow affected the population of their host fish. Esox lucius (northern pike) are presently common in Shell Lake (personal observation), but quantitative data on this and any other fish species present are not available. A. grandis has relatively low host specificity (Trdan and Hoeh 1982; Fuller 1974; Kat 1984), but no member of the Esocidae has been verified as a host. However northern pike have been found to be parasitized by another unionid (Amblema plicata : Coker et al. 1921; Wilson 1916), so we tentatively reject an impact on the fish population as a mechanism for the observed decline of A. grandis in Shell Lake. Further evidence, perhaps including bioassays of juvenile mussels cultured in the laboratory (Isom and Hudson 1982; Hudson and Isom 1984), is required to verify the hypothesis that hydrocarbon pollution has directly affected the survival of juvenile mussels.

Limitations of the "Impact" Interpretation

There are several relevant limitations to interpreting the differences in density and length-frequency distribution between 1973 and 1986 as a result of hydrocarbon pollution. We have a sub-optimal design for detecting an impact (sensu Green 1979), because the presumed

impact (hydrocarbon pollution) has already occurred, there is no control area, and therefore the "impact must be inferred from temporal change alone" (Green 1979). Clearly any natural change in the population between 1973 and 1986 could have been mistakenly interpreted as a detrimental impact on the population, although the direction of the change (a declining population) is consistent with the impact interpretation.

We have no "among year" or "among lake" replication, meaning that whatever differences in the A. grandis population that we observed between 1973 and 1986 could have just as easily been present between two consecutive years in one lake or in two lakes sampled in a given year, each with similar levels of hydrocarbon pollution. Such "pseudoreplication" (Hurlbert 1984) is impossible to avoid when specific questions or limited data reduce the sampling frame.

Finally, any difference in the techniques used by the two samplers (RHG in 1973; RCB in 1986) may have "created" the observed differences between data from the two years of sampling. We tried to minimise this by matching the sampling method but hand-sampling with SCUBA is bound to result in some human bias. The pronounced differences in density and length-frequency distributions for the two years of sampling are probably greater than any sampling bias could introduce.

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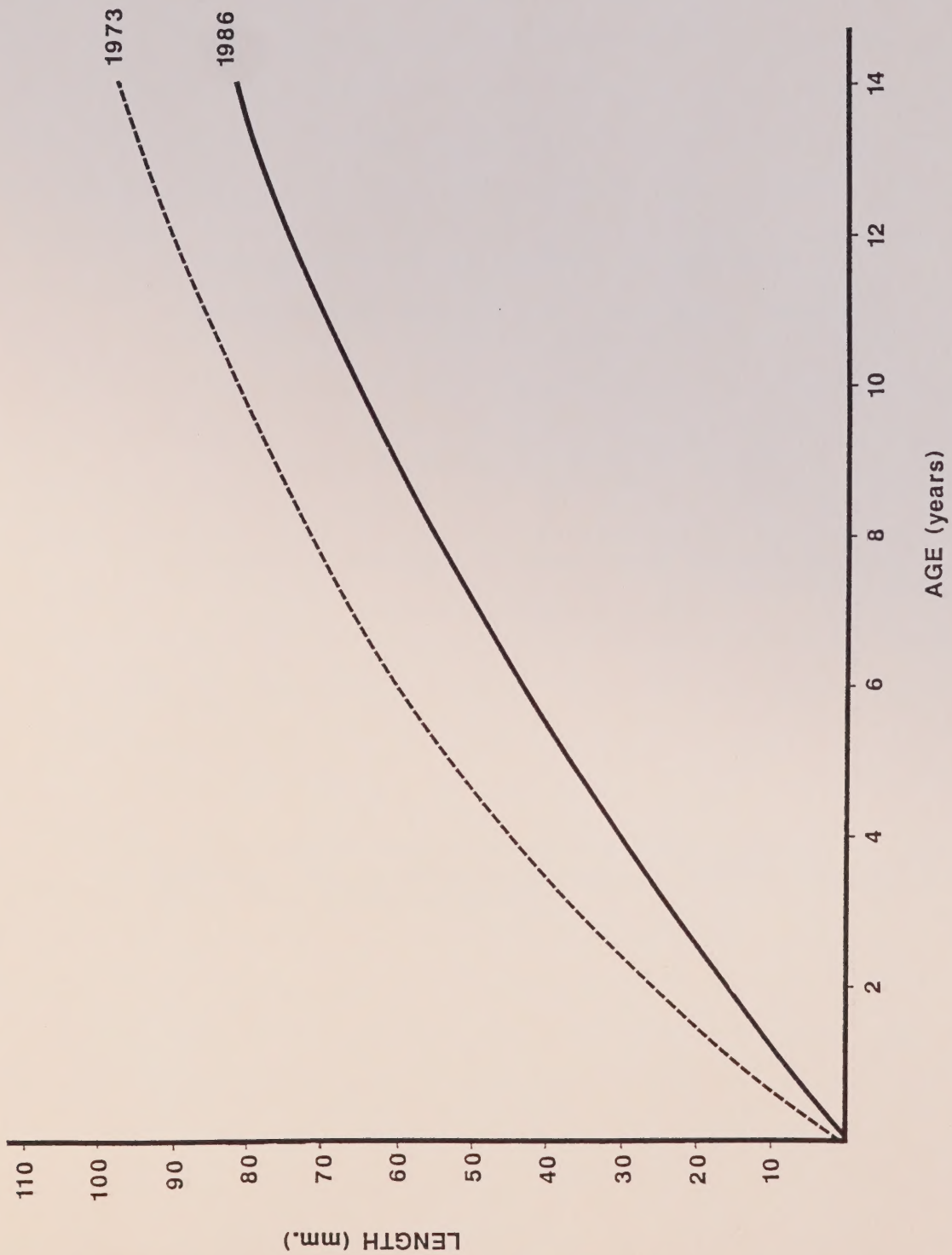
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Table 1. Number of replicates in each cell for the factorial ANOVA of log(density). Each replicate was one pair of 2m² circular plots.

Year	Depth	
	<1.5m	>1.5m
1973	10	14
1986	8	10

Table 2. Length class distribution of mussel shells in relation to depth and year. Percentage of mussels in a given length class for a particular depth and year is given in parentheses.

		Length Class				
		<60mm	60-74mm	75-89mm	>90mm	TOTAL
1973						
	≤2.0m	4 (0.6)	279 (39.7)	398 (56.7)	21 (3.0)	702
	>2.5m	4 (1.3)	145 (45.7)	133 (42.0)	35 (11.0)	317
1986						
	≤2.0m	8 (7.6)	9 (8.6)	69 (65.7)	19 (18.1)	105
	>2.5m	24 (7.7)	36 (11.5)	196 (62.6)	57 (18.2)	313



Freshwater clams as monitors of variation in
environmental acidity and trace metal levels

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Summary

We collected freshwater clams (Elliptio complanata) from an acid sensitive and a circumneutral lake in south-central Ontario and compared tissue metal concentrations. Clams from the circumneutral lake had greater concentrations of Zn and Mn and lower concentrations of Cu and Cd than clams from the acid sensitive lake. Clam size and/or age successfully predicted tissue metal levels, suggesting that long-term clam biomonitoring studies should control for size and age variability.

We examined the effects of environmental acidity on E. complanata shell morphology and concluded that differences in shell size and shape were a function of within-lake variation in water turbulence. Therefore the use of shell morphology as an indicator of environmental acidification requires prior consideration of within-lake variability in shell form.

Clam shells were not useful monitors of environmental trace metal levels.

Introduction

Molluscs have, for over 10 years, been used to monitor contaminant levels in the marine environment (Goldberg et al. 1978). They accumulate and concentrate a wide range of environmentally available pollutants, and they are widely distributed both globally and in terms of the habitats that they occupy (Green et al. 1985). In recent years, freshwater clams have been used to monitor variation in environmental contaminant levels through in situ (Forester 1980; Imlay 1982; G.L.I. 1984; Pugsley et al. 1985) and transplantation (Kauss et al. 1981; G.L.I. 1985) studies. It is uncertain, however, whether freshwater clams exhibit size- and age-specific contaminant accumulation patterns similar to their marine counterparts (Boyden 1977; Romeril 1979; Cossa et al. 1980). This must be determined before freshwater clams are extensively used as biomonitors. In this study we examine size- and age-specific patterns of trace metal concentrations in the freshwater clam Elliptio complanata.

The bioavailability of many trace metals is enhanced by increasing environmental acidity (Campbell et al. 1983; Campbell and Stokes 1985; Stokes et al. 1985; Kelso et al. 1986). In acid stressed, poorly buffered waters, bioaccumulation of trace metals has been reported in clams (Forester 1980; Graney et al. 1984). We will compare tissue metal concentrations in E. complanata from an acid sensitive and a circumneutral lake. The relative importance of these patterns will be examined by comparing them to the size- and age-specific relationships.

Although clam tissues are useful for monitoring short-term fluctuations in aquatic contaminant levels, clam shells may be useful as indicators of long-term water quality patterns. It has been suggested that variation in environmental acidity can cause variation in shell shape (Mackie and Flippance 1983) and growth rate (Green 1980; Singer 1981) because calcium, which is necessary for shell growth, is often less available in low alkalinity environments. We examined this hypothesis by sampling E. complanata from four small lakes that vary in alkalinity and compared within-lake to between-lake variation in shell morphology.

Marine clam shells can retain a record of contaminant levels over many years (Fang and Shen 1984), so clam shells may provide a more long-term and irreversible system for monitoring changes in environmental levels of trace metals than clam tissues. We utilized the electron probe and the secondary ion mass spectrometer to assess trace metal levels in thin sections of freshwater clam shells sampled from known contaminated areas.

Methods

Age- and size-specific trace metal patterns

We collected 50 E. complanata, including as wide a size range as possible, from the southwest corners of Beech Lake and Tock Lake using SCUBA. These lakes are located in south-central Ontario on the Precambrian Shield (Fig. 1). Although morphologically similar, the lakes vary greatly in alkalinity

with total inflection point alkalinities for Tock and Beech of 22 and 153 ueq/l respectively. Shell lengths were determined and used as a measure of clam size. Clam tissues were dissected into gill and remaining visceral mass (body), then dried and digested in concentrated nitric acid, and analyzed using flame spectrophotometry for levels of Cu, Cd, Zn, and Mn. Shells were aged by the thin-section method (Clark 1980).

Analysis of variance (ANOVA) was used to compare differences in gill and body trace metal concentrations between the lakes. Analysis of covariance (ANCOVA) was used to compare the relationships between clam age and length and tissue metal concentration. Tissue metal concentration and length data were log-transformed, prior to analysis, to reduce error variance heterogeneity.

Within- and between-lake variation in shell form

We collected 25 E. complanata using SCUBA from a low (southwest) and a high (northeast) exposure site (i.e. water turbulence) in each of four small lakes (Figs. 1 and 2). Only old individuals were collected to eliminate age structure as a confounding factor. Shell length, height, width and weight were determined (Fig. 3). These data were log-transformed to linearize allometric relationships between the variables.

A model II ANOVA estimated the proportion of the total variance of each variable due to variation between the four lakes, between exposure levels within the lakes and between individual clams within the sites.

Shell analysis for trace metals

Thin sections of clam shells were prepared for the electron probe following the methods of Lutz (1981). As, Hg, Pb, Cd, Ca, Sb and Zn levels can be determined by the characteristic x-rays which are emitted from the thin section when an electron beam is focussed on it. The secondary ion mass spectrometer, which is a more sensitive instrument than the electron probe, was also used to determine levels of the same metals in the thin sections.

Results

Age- and size-specific trace metal patterns

Trace metal concentrations in both gill and body tissues differed between Beech Lake and Tock Lake clams (Table 1). Tock Lake clam tissues were higher in Cu and Cd and lower in Zn and Mn than Beech Lake clam tissues ($P < 0.05$). There was not any strong relationship between clam size and age possibly because of the lack of extremely small individuals in the sample. Size and age could therefore be used as independent covariates in each analysis. Each model (Table 2) was successful in predicting tissue trace metal variability from clam size and age.

Within- and between-lake variation in shell form

In Green, Beech, Bark and Tock Lakes, clams from the high exposure site had longer, taller, wider and heavier shells than those from the low exposure site (Table 3). Components of variance computed from the model II ANOVAs revealed that much

more of the variation in shell morphology resulted from differences between exposures rather than differences between lakes (Fig. 4).

Shell analysis for trace metals

The electron probe analyses showed no detectable levels of As, Hg, Cd, Pb or Zn in the shells examined. The secondary ion mass spectrometer detected only major shell elements (i.e. Ca, C, Sr, Na, Fe, Mn) which were in part per thousand concentrations.

Discussion

Age- and size-specific trace metal patterns

Cu, Cd, Zn and Mn are known to increase in geochemical mobility with increasing acidification (Campbell et al. 1983) and are generally in greater concentrations in Ontario non-buffered versus buffered waters (Forester 1980). Tock Lake is acid sensitive and has low buffering capabilities while Beech Lake is circumneutral and thus possesses the ability to buffer fluctuating levels of acidic input. Analyses of a small number of water samples from these lakes (unpub. data) suggest that absolute levels are generally greater in Tock Lake. However, Beech Lake has greater Mn and Zn levels relative to Cu and Cd than does Tock Lake. Concentrations of metals in the clams showed similar patterns, as Beech Lake clams had higher Mn/Cu, Mn/Cd, Zn/Cu and Zn/Cd ratios. These results closely resemble

those of Servos (pers. comm.; Freshwater Institute, Winnipeg Manitoba) who examined bioaccumulation of metals by E. complanata during acidic snowmelt. In his study, Zn concentrations increased relative to Cd concentrations in gill and body tissues as environmental acidity decreased.

There can be competition between metals for a limited supply of ligand (binding substrate) within marine mollusc tissue, and the competitive nature of metals for ligands can be altered depending on environmental metal levels (Mason and Simkiss 1983). It is thus possible that higher absolute metal levels (i.e. in Tock Lake) enhance the competitive nature of Cu and Cd for binding substrate while lowering that of Zn and Mn. Competition between trace metals for binding substrate in E. complanata tissues has been reported by Tessier et al. (1984) who determined that Fe competes with Cu and Zn. They suggested that Fe plays a protective role as it is the principal factor controlling the dissolved trace metal concentrations to which the clam is exposed.

Gill tissue metal concentrations were often twice that of body tissue. Gill concentrations may be overestimated and may not reflect biologically available metals, because available and non-available metals can adhere to mucous covered gill surfaces. Including gills in whole body analyses may therefore be inappropriate, because they could decrease the predictive power of the model by increasing body tissue trace metal variability.

Size- and age-specific trace metal relationships were different for each tissue type and each metal. This type of variability is common in marine bivalves (Boyden 1977; Romeril

1979; Cossa et al. 1980; Popham and D'Auria 1983). There are many factors which could enhance variability in these patterns such as feeding habits, reproductive state and condition factors. However, physiological explanations for the observed patterns are not within the scope of this paper. Size and age are important factors which contribute to tissue trace metal variability. Therefore, investigators who wish to compare E. complanata metal concentrations should standardize clam size and age. This would especially apply to clam biomonitoring studies where comparisons are made among sites and years.

In only two cases (gill Cu and body Cd) did lake interact with clam size and/or age in modelling tissue trace metal variability. Buffering capability, which is the primary difference between the two lakes, is therefore not very important in influencing size- and age-specific patterns. However, with only two lakes in the analyses, we have very little power to detect such an influence.

Within- and between-lake variation in shell form

Exposure affected variation in shell morphology similarly in all four lakes. Clams from low exposure sites were smaller and lighter than those from high exposure sites. These characteristics may be adaptive because small, light shells would preclude sinking in low exposure, soft silt areas while large, heavy shells would prevent dislodgement by wave action in high exposure, sandy areas.

Some researchers have suggested that variation in alkalinity

can cause variation in clam growth patterns (Green 1980; Singer 1981; Mackie and Flippance 1983). However, interpretation of those studies is often difficult because within-lake variation in growth patterns was not considered. In this study, much more of the total variation in shell form was due to within-lake between-exposure differences rather than between-lake differences. The primary difference between the four lakes is alkalinity; therefore alkalinity has little to do with variation in E. complanata shell form. Our results indicate that within-lake variation in shell form must first be assessed or controlled by the study design, before among-lake variation is examined.

Shell analysis for trace metals

The electron probe has a maximum sensitivity of 200 ppm, however, it could not detect As, Hg, Cd, Pb or Zn in the shells examined. Similar results were obtained from the more sensitive secondary ion mass spectrometer. Body tissue analyses of the same specimens had shown significant As, Hg and Pb concentrations (Metcalf and Mudrock 1985).

Conclusions

Freshwater clam tissues can be a useful tool for monitoring in situ levels of biologically available trace metals in areas affected by acidic precipitation once size and age effects are controlled for. Before large scale transplantation studies are designed, heritability of trace metal uptake rates should be examined. For example, Hinch et al. (1986) concluded that growth

rates of the unionid clam Lampsilis radiata were a function of the population source (in a field transplant experiment). Other physiological processes which may be related to trace metal uptake may also be a function of the population source. A three-way reciprocal transplant experiment has been conducted using E. complanata and three lakes which represent the "degree of acidification" gradient. These data are presently being analysed. Electrophoretic studies in allozyme frequencies are being done, to investigate the role of genotype in trace metal uptake.

Although clam shells have proven to be useful monitors of contaminant levels in marine environments, they were not useful for monitoring levels of trace metals at the environmental concentrations observed in this study. Shell morphology is not a good predictor of environmental acidity but probably reflects site-specific water turbulence.

Acknowledgements

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Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc Elliptio complanata in a mining area. Canadian Journal of Fisheries and Aquatic Sciences, 41, 1463-1472.

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Table 1. Mean metal concentrations in p.p.m (SD) in gill and body tissues of E. complanata from Beech Lake and Tock Lake. Each Mn concentration is x 102.

		Beech Lake		Tock Lake	
gill	Cu	13.28	(5.69)	18.51	(7.83)
	Cd	10.10	(4.34)	12.78	(4.49)
	Zn	371.32	(108.75)	273.81	(72.80)
	Mn	140.80	(40.30)	104.90	(32.70)
body	Cu	7.29	(1.83)	10.29	(6.95)
	Cd	10.98	(5.82)	14.51	(4.62)
	Zn	155.50	(43.87)	134.17	(63.15)
	Mn	42.20	(19.60)	23.50	(12.00)

Table 2. ANCOVA models which predict tissue trace metal variability from clam size and age in Beech Lake and Bark Lake.

dependent variable: gill Cu
model $r^2=.34$

source of variation	P
length	.106
age	.001
lake	<.001
length * age	.521
length * lake	.250
age * lake	.462
length * age * lake	.014

dependent variable: gill Cd
model $r^2=.18$

source of variation	P
length	.002
age	.443
lake	.021
length * age	.457
length * lake	.737
age * lake	.170
length * age * lake	.456

dependent variable: gill Zn
model $r^2=.25$

source of variation	P
length	.759
age	.003
lake	<.001
length * age	.642
length * lake	.378
age * lake	.278
length * age * lake	.403

dependent variable: gill Mn
model $r^2=.23$

source of variation	P
length	.923
age	<.001
lake	<.001
length * age	.717
length * lake	.634
age * lake	.303
length * age * lake	.692

dependent variable: body Cu
model $r^2=.20$

source of variation	P
length	.049
age	.073
lake	.004
length * age	.043
length * lake	.532
age * lake	.885
length * age * lake	.241

dependent variable: body Cd
model $r^2=.50$

source of variation	P
length	<.001
age	.300
lake	<.001
length * age	.033
length * lake	.015
age * lake	.602
length * age * lake	.887

dependent variable: body Zn
model $r^2=.20$

source of variation	P
length	.035
age	<.001
lake	.005
length * age	.739
length * lake	.598
age * lake	.952
length * age * lake	.727

dependent variable: body Mn
model $r^2=.42$

source of variation	P
length	.119
age	<.001
lake	<.001
length * age	.911
length * lake	.549
age * lake	.261
length * age * lake	.406

Table 3. Means (SD) of shell characters from low and high exposure sites in the study lakes. In all groups n=25.

Site	Length (mm)	Height (mm)	Width (mm)	Weight (g)
Tock low	66.0 (5.4)	33.2 (2.7)	17.7 (1.9)	9.6 (2.9)
Tock high	74.1 (5.8)	37.2 (3.0)	19.6 (2.1)	14.1 (3.6)
Bark low	59.4 (4.5)	28.6 (1.8)	17.8 (1.5)	8.1 (2.0)
Bark high	64.2 (3.1)	30.9 (1.5)	19.4 (1.2)	10.8 (2.1)
Beech low	63.3 (6.3)	31.6 (3.5)	16.7 (2.4)	8.5 (2.8)
Beech high	72.1 (6.3)	36.6 (3.1)	19.7 (2.2)	13.6 (3.7)
Green low	63.0 (8.6)	31.1 (4.2)	16.2 (2.6)	8.8 (4.5)
Green high	66.9 (7.7)	33.1 (3.8)	17.7 (2.5)	11.0 (4.7)



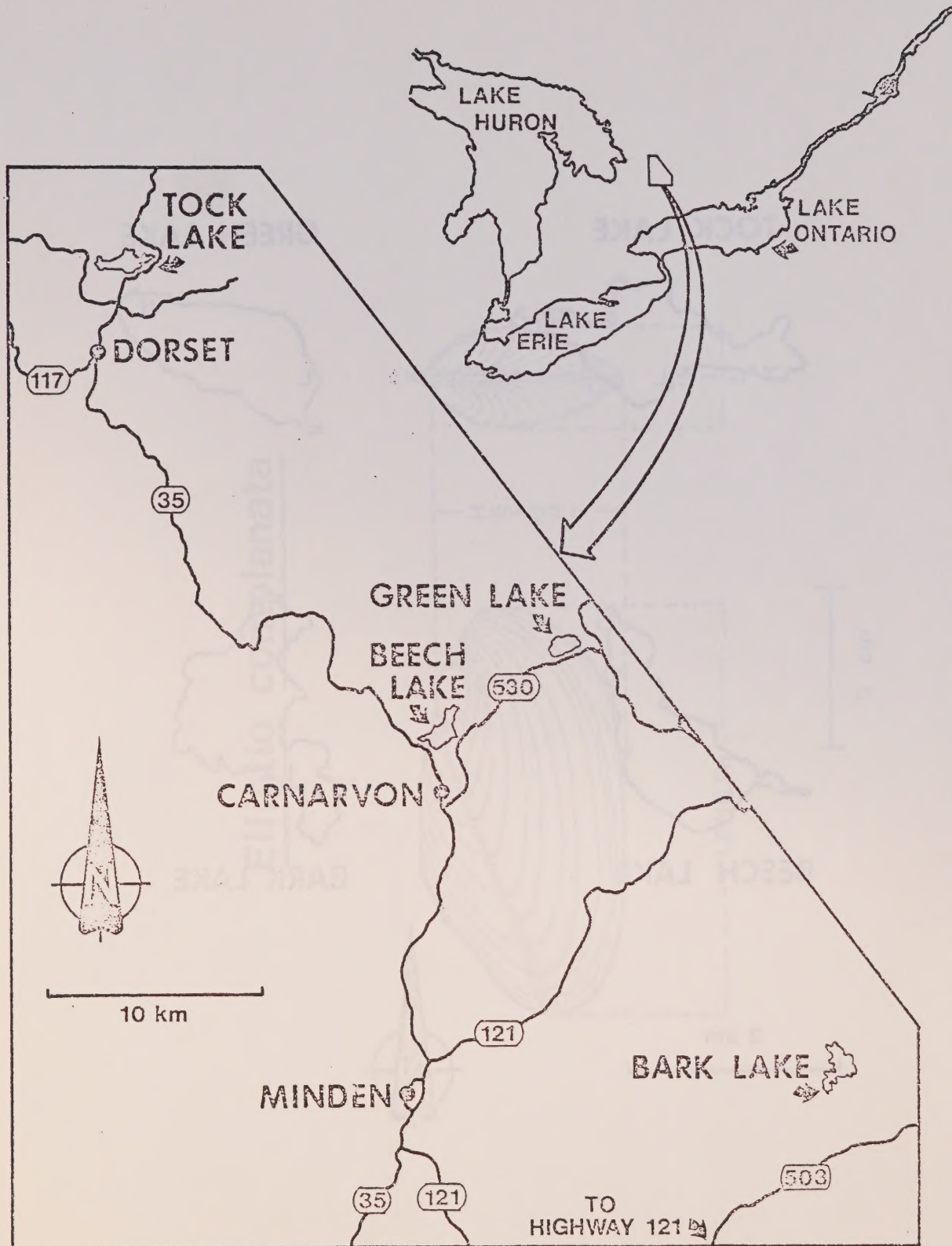
List of Figures

Fig. 1. Location of study area in south-central Ontario and detailed locations of lakes in relation to provincial highways and towns.

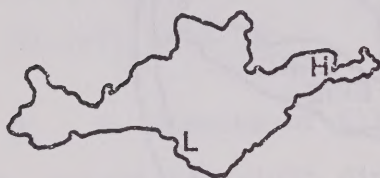
Fig. 2. Four study lakes with the low (L) and high (H) exposure sites in each lake.

Fig. 3. A drawing of Elliptio complanata (Unionidae) showing length, height and width dimensions.

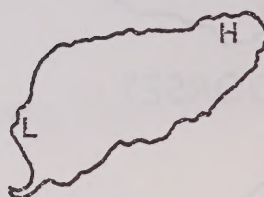
Fig. 4. Proportions of total variance in shell height, length, weight and width due to variation among the four lakes, between exposure levels within the lakes and among individual clams within the sites.



TOCK LAKE



GREEN LAKE

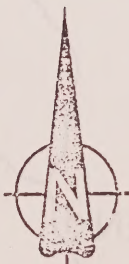


BEECH LAKE

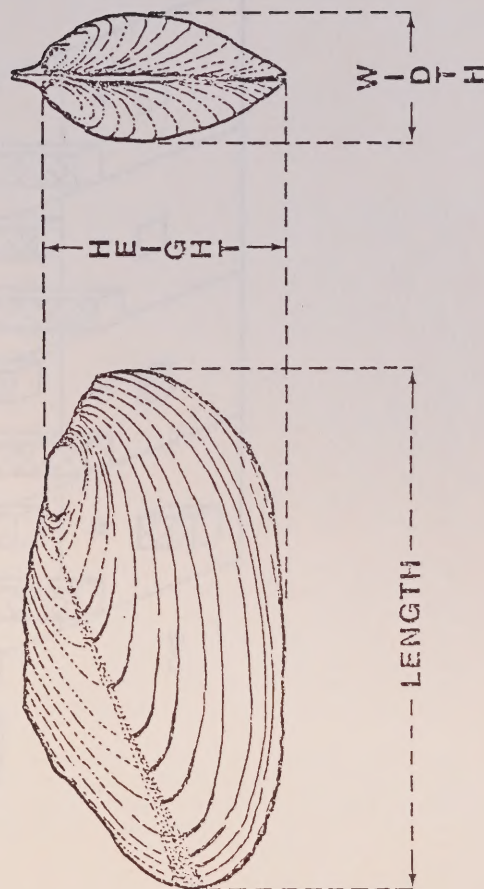


BARK LAKE

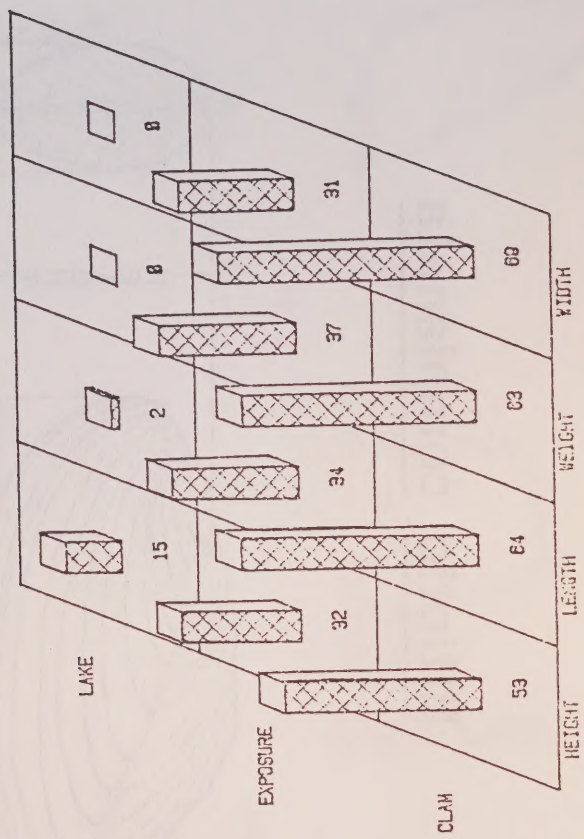
2 km



Elipio complanata



3 cm



BIVALVE MOLLUSCS AS RESPONSE SYSTEMS FOR MODELLING SPATIAL AND TEMPORAL ENVIRONMENTAL PATTERNS

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ABSTRACT

Organisms respond to their environments at different hierarchical levels, ranging from the community level (species abundance patterns) to the cellular and genetic levels. One often wishes to model these responses as functions of spatial and temporal variation of environmental variables, either for the purpose of describing and understanding the processes which are operating or for the purpose of predicting the environmental state given an observed spectrum of biological response. The latter purpose obviously relates to environmental monitoring and other practical applications.

For modelling the biological response to patterns of environmental variation, one needs a suite of organisms having the following properties: (1) ubiquitous in the environments of interest; (2) responsive at the various hierarchical levels (community, population, physiological, cellular, genetic) to environmental change; (3) long-lived and sedentary in habit; (4) possessing skeletal parts which incorporate, and retain after the individual's death, annual patterns of the physical and chemical environment; (5) suitable for handling and for experimental studies in the laboratory; and (6) relatively well understood genetically.

One group of organisms which has proved particularly suitable and meets the above criteria is the bivalve molluscs. They are ubiquitous, sedentary, and responsive to their environment at both the micro- and macro-geographical scales and at all levels of biological organization. Many species can be aged using the periodic rings in their shells. The chemical composition of different layers of the shell can be used as records of their exposure to past environments. Methods for laboratory culture and genetic studies are well established. A number of species are polymorphic at many loci and have been extensively used in population genetic studies.

Models will be discussed which relate the responses of bivalve molluscs, at the community and population, as well as the physiological, behavioural and genetic levels, to environmental variation in space and time. We will present a critique of studies to date and suggest approaches for those to be undertaken in the future.

INTRODUCTION

The modelling of biological response

All organisms respond to environmental stimuli and stresses in a hierarchical manner which has been described by Slobodkin (1968) as evolutionarily

adaptive. The initial response tends to be behavioural (e.g. more rapid ventilation, or movement away from a negative stimulus), and then, if the stress has not been eliminated, biochemical and physiological (e.g. increased metabolic rate). Next affected are population parameters, such as fecundity, mortality and dispersal, that may ultimately affect the genetic composition of the next generation. Severe reduction in the populations of some species will result in community composition change, which may be irreversible (e.g. Kerr, 1977). The most important point is that no one biological response variable can suffice; a battery of responses covering different hierarchical levels and correspondingly different response periods may be required, depending on the time scale(s) of interest.

The criterion and predictor variables must be connected to each other, and we do this by using some kind of model. The model may be verbal and non-quantitative ("Within a few hours of the chemical spill, all the shellfish in the bay died."), or it may be based on a planned experiment and expressed with great statistical sophistication. In either case certain assumptions must be satisfied or the 'model statement' will be of little value: (1) the criterion variable (e.g. death of shellfish) must convey some useful meaning, such as economic loss or ecological damage in an *a priori* healthy biological community; (2) there must be some plausible causal mechanism that could have brought about the response as a result of the change described by the predictor variable; (3) any predictor variable which might have influenced the criterion variable must have been included in the model (i.e. nothing must be omitted that might be relevant); and (4) some kind of control situation existed.

The change or variation described by the predictor variable would usually be temporal, as would the biological response. However, the impact of environmental factors on biota can also be inferred from the spatial pattern. 'Temporal variation only' data would lack a spatial control, although a temporal control ('before' as opposed to 'after') may exist. On the other hand, 'spatial pattern only' data lack a temporal control. Therefore, both lack one of the two kinds of control, and are in that sense 'suboptimal' in contrast to an 'optimal design' (Green, 1979a) which combines both spatial and temporal variation, including controls, in a balanced design. Genuine replication is difficult to obtain in observational studies (Manly and Wright, 1982; Hurlbert, 1984; Green, 1984a, 1985), which has serious consequences for statistical models. This problem is discussed later.

We model the coupling of environmental stimulus to biological response. We build the model to fit observed data, striving for maximum parsimony, but adequate sufficiency. Next we validate the model, or perhaps modify it, by predicting from new observations on the predictor variables to new observations on the criterion variables. This stage is often best performed, or accompanied, by a controlled experiment carried out in the field if possible, or in the laboratory if necessary. Finally, we use the validated model with practical objectives in mind, predicting the impact or nature of environmental change using the modelled biological response as an index.

Bivalve molluscs as response systems

The following appear to be the requirements of any group of organisms which would be used as a biological response system for modelling spatial or temporal patterns of environmental variation, particularly in nearshore marine environments: (1) a large number of species should be present in abundance in all regions within those environments; (2) they should generally be responsive at the different hierarchical levels to environmental change; (3) at least some common and widely distributed taxa within the group should be long-lived and sedentary in habit; (4) they should produce hard parts which record the environmental state at the time of their formation by the living organism, and survive the death of the organism for a length of time much greater than the organism's life span; (5) they should be easy to sample in the field and suitable for holding and for experimental studies in the laboratory; and (6) they should be relatively 'well-worked' genetically.

Do bivalve molluscs satisfy these requirements? Cunningham (1979) gives a similar list and suggests that they do. Something like 31 super-orders of bivalve molluscs exist, containing about 420 genera in total (Morton, 1964), with about 15 000 species which are distributed world-wide throughout most marine, estuarine and freshwater environments. It is a taxonomically well-worked group, with most species identifiable from the shells alone. Even the larvae of marine bivalves are sufficiently well known and described, that common species can usually be identified from field collections (Chanley and Andrews, 1971), and use of the scanning electron microscope offers great promise (Turner and Boyle, 1974; Calloway and Turner, 1978).

The sensitivity of bivalves to environmental stimuli is now well documented. Growth rates and temperature tolerances often show clear geographic patterns related to factors such as salinity (Lubinsky, 1967) and temperature (Kennedy and Mihursky, 1971, 1972). Very fine-scale micro-habitat environmental variations can also be mapped. For example, variation in tidal height and exposure to wave action, over small distance, can influence mortality rate (Green and Hobson, 1970), shell morphology (Newkirk and Doyle, 1975) and gene frequencies (Koehn et al., 1973; Newkirk and Doyle, 1975; Levinton and Fundiller, 1975). A number of reviews have discussed aspects of physiological response in marine molluscs (Bayne et al., 1981), use of bivalves in heavy metal monitoring (Cunningham, 1979), and the use of mollusc shells as indicators of their past environment (Imlay, 1982). Responses to environmental quality have been reported at the physiological/behavioural level (gill ciliary beat rate, Paparo and Sparks, 1977), for reproduction (Bayne, 1975), and for growth processes (shell size and shape, Green, 1972). Papers reporting responses to temperature are: at the community level (pelagic larvae as paleoclimatic indicators, Lutz and Jablonski, 1978b); at the physiological level (shell calcification/dissolution processes, Davies and Crenshaw, 1971; Storr et al., 1982); and mortality rate (Incze et al., 1980). Papers reporting responses to salinity or other components

of water chemistry are: at the behavioural level (response to heavy metals, Salanki and Varanka, 1976); at the physiological level (uptake of heavy metals, Phillips, 1976); at the genetic level (response to salinity, Koehn et al., 1976); and at the level of growth processes (Davies and Crenshaw, 1971). Physiological responses to oil are described by Gillfillan et al. (1977) and Jeffries (1972).

Many bivalves are long-lived. Some unionid species live for 40–60 years (Imlay, 1982). In the marine environment slow growth and longevity tend to be associated with deeper or colder environments, e.g. *Arctica islandica* up to 90 years or more (Thompson et al., 1980), *Macoma calcarea* and *Mya truncata* up to 30 years (Peterson, 1978; Green et al. unpublished), *Macoma balthica* up to 24 years (at 35 m in the Baltic, Segerstråle, 1960), and *Spisula solidissima* up to 25 years (at 60 m in the North Atlantic, Jones et al., 1978, 1983). Perhaps a life span of a decade is typical for nearshore temperate marine environments.

Most post-larval settlement bivalve molluscs are sedentary. For species that are not attached to hard substrates as adults, most movements are seasonal and on the scale of a few meters to a few hundred meters, even for lotic unionid species (Coker et al., 1919).

Gene flow within and among populations is not usually restricted by the movements of adults. Pelagic larvae of many marine species, and the glochidia of freshwater unionids which attach to fish hosts, ensure wide genetic dispersal. Fujio et al. (1983) surveyed genetic variation in 25 species of marine molluscs (of which 23 were bivalves representing nine families) and concluded that they were generally more variable than marine teleosts, which certainly are characterized by adults capable of moving great distances during their life span. It is interesting that the pisidiids of fresh water, which lack a pelagic larva, are ovoviviparous and capable of self-fertilization, are reported to have relatively low genetic variability (Hornbach et al., 1980). Four *Sphaerium* species average 8% of loci polymorphic compared with 41% for marine molluscs (from Fujio et al., 1983), and 0.4% of loci heterozygous compared with 13% for marine molluscs.

It is possible to culture a large number of bivalve species with ease and use them in laboratory studies. These include mussels, oysters (Haley et al., 1975) and *Mercenaria* (Castagna and Kraeuter, 1981), as well as the freshwater Unionidae (Isom and Hudson, 1982; Hudson and Isom, 1984) and Pisidiidae (Mackie et al., 1978). Also, a number of bivalve species have been used extensively in genetical studies, particularly at the population level (see reviews by Longwell, 1976; Newkirk, 1980).

The potential of the bivalve mollusc shell as a record of the environment which existed during its formation has long been recognized. The bivalve shell can record the environment in four different ways: growth rate, shell form, shell mineralogy and microstructure, and shell chemistry. Rate of growth is usually estimated from the spacing between annual, or other periodic, rings. Major review papers Haskin (1954), Clark (1968), Rhoads and Panella (1970), and Lutz and Rhoads (1980). Marking of individuals and

then following their growth over one or more years has been done in a number of cases (e.g. Chamberlain, 1931; Frank, 1965; Peterson et al., 1983). It is now possible to use stable isotopes to validate age estimates from shell rings (e.g. Jones et al., 1983). Variation of mollusc shell form as a function of environment has been described for many species, e.g. periwinkles (Newkirk and Doyle, 1975) and oysters (Groue and Lester, 1982), fresh water mussels (Green, 1972) and fingernail clams (Bailey et al., 1983). Fresh water unionacean shells also have great intraspecific variability (see Tevesz and Carter (1980a) for a review). Environmental influence on shell mineralogy has been considered mostly with regard to whether CaCO_3 is deposited as calcite or aragonite. However, that is not a simple matter, because of taxonomic diversity in the physiological processes involved (Carter, 1980). For chemical and isotopic composition of shells as an indicator of environment, Rosenberg (1980), Rye and Sommer (1980), and Imlay (1982) provide recent reviews. The best recent reference for all aspects of the use of shells as "biological records of environmental change" is Rhoads and Lutz (1980).

REVIEW OF THE USE OF BIVALVES AS RESPONSE SYSTEMS

In this section we will critically review previous work. Studies fall into three categories: (a) community and population studies; (b) studies on physiology, behaviour and genetics; and (c) studies on shell structure and chemistry.

(a) Community and population studies

The community level of response to environmental patterns has probably been recognized the longest, and yet it is still the most contentious and difficult to apply unambiguously. The attraction of, and the difficulties with, community species composition as a response variable is that it is the most biologically complex and integrated response. As Cairns (1983) puts it, the responses "are so complex that they could not be predicted from a detailed examination of the isolated component parts", and yet often "an assumption is made that responses at levels above single species can be reliably predicted with single species tests". The most pressing need "is not further perfection of single species tests, but rather the development of parallel tests at higher levels of organization." That is all very well, but one must ask why this pressing need has not already been met. There is no lack of evidence that community composition is responsive to environmental impacts (e.g. Linden, 1977; Loi and Wilson, 1979; Linden et al., 1979; Kat, 1982; Elmgren et al., 1983).

We would suggest five reasons why community response is a difficult variable to work with in practice; all are related to the complexity of the response. First, the observed shift in species composition often appears

straightforward as a superficial description but, on closer examination, the response is less clear, as we begin to see the complexities of the other response levels which have been integrated to produce the community response. There are population phenomena, such as composition (Levinton and Suchanek, 1978), age structure effects (Dow, 1978; Bauer, 1983), and genetic composition (Green et al., 1983), operating over space and time. In some cases, a supposed indicator species may actually turn out to be a suite of sibling species (e.g. Grassle and Grassle, 1976).

Second, community and population responses to environmental stress are often very non-specific. They may not be responses to the levels of environmental factors at all, but rather to their variability (e.g. Jackson, 1972). Species populations that are adapted to unpredictable environments are often more genetically variable and more resistant to environmental stresses (e.g. Levinton and Suchanek, 1978; Green et al., 1983). Similarly, responses to human-derived impacts often 'map' the genetically based tolerance to variability of the natural environment (Fisher, 1977; Zuim and Mendes, 1980).

Third, 'community change' as a hypothesis implies a null hypothesis of no change, or stability, or persistence in some sense. There is no general agreement on the criteria for stability of communities, and indeed there is a real question of whether most natural, unimpacted marine communities are stable in any meaningful sense. Perhaps the only meaningful error term for testing the hypothesis of impact-caused community change is natural year-to-year variation (Green, 1984a, 1985). This means that long-term studies are necessary or, alternatively, short-term experiments with artificial communities (e.g. Sutherland and Karlson, 1977).

Fourth, because of their complexity community level studies are usually carried out as observational 'experiments' in the field, and therefore they have severe inherent limitations. Because of the impossibility of randomly allocating replicates to the treatment and control conditions (Manly and Wright, 1982; Hurlbert, 1984; Green, 1984a), errors may be correlated and some variables may be confounded by the design. In many cases confounding is obvious and unavoidable, for example along estuarine or distance-from-shore gradients. Human-derived impacts are also often correlated with these gradients. The greatest danger is the possibility of undetected confounding, and the burden of proof lies with the investigator. The only adequate precaution is randomization in a controlled experiment, hence the inadequacy of any observational study.

Fifth, the response of a natural community to environmental variation is usually complex and multivariate, difficult to describe and even more difficult to analyze statistically. Diversity indices (Hurlbert, 1971; Goodman, 1975; Green, 1979a) and indicator species (Hart and Fuller, 1979) have been used to reduce the multivariate response to one or a few key variables. Multivariate statistical approaches offer great promise (Green, 1980, 1984a), but are still used mostly for an a posteriori description, and in many cases for salvage operations where careful planning and adequate sampling or experimental design were not employed.

(b) *Physiology, behaviour and genetic studies*

Responses at this level fall into one of two categories. The first deals with resistance or survival at environmental extremes (e.g. LD₅₀), while the second includes the capacity for adaptation or maintenance of rate functions at optimum levels over the normal environmental range. The capacity to adapt may result from decreasing energy expenditure (energy-conserving) or increasing energy inputs (energy-supplementing). Mann (1978) compared some indices of conditions (morphometric, biochemical and physiological) that have been used to measure the effects of stresses. These include the ratio of meat content to potential maximum size (gravimetric index), and some biochemical indices, such as percentage carbohydrate, carbohydrate/protein ratio, C/N ratio, percentage organic (100-ash) content, and the ratio of O₂ consumption rate to ammonia excretion rate. It is hoped that modern techniques will provide more sensitive responses to stress at the biochemical, physiological and genetical levels. The capacity for adaptation, reflecting homeostatic buffering following exposures to adverse environmental conditions, is probably species-specific. Therefore, species to be used for environmental monitoring should be abundant, phenotypically and genotypically variable, and easy to handle and to experiment with in the laboratory. For most aquatic systems molluscan species satisfy these requirements. In particular, mussels (e.g. *Mytilus* spp.) have been used extensively for such programs (Keckes et al., 1968, 1969; Lee et al., 1972; Bayne, 1976; Phillips, 1976; Stureson, 1976; Bayne et al., 1976, 1979; Cunningham, 1979; Freeman and Dickie, 1979; Cossa et al., 1980; Davies and Pirie, 1980; Zuim and Mendes, 1980; Ouellette, 1981; Aarset and Zachariassen, 1982; Poulsen et al., 1982; Arimoto and Feng, 1983; Ekelund et al., 1983; Fischer, 1983; Dickie et al., 1984). Some of the other molluscan species used for such monitoring programs include *Macoma* spp. (Wilde, 1975; Linden, 1977; Eldon and Kristoffersson, 1978; Linden et al., 1979; McGreer, 1979; Clement et al., 1980; Eldon et al., 1980; Shaw and Wiggs, 1980; Stekoll et al., 1980; Langston, 1982; Elmgren et al., 1983), oysters (Davis and Hidu, 1969; Cunningham and Tripp, 1973; Frazier, 1976; Ellis et al., 1981; Watling, 1982), *Mya* spp. (Gillfillan et al., 1977; Appeldoorn, 1981; Walker et al., 1981; Macdonald and Thomas, 1982), and other shellfishes and clams (Foehrenbach, 1971; Jeffries, 1972; Nielsen and Nathan, 1975; Smith et al., 1975; Paparo and Sparks, 1977; Cunningham, 1979; Ogata and Miyake, 1981).

Overall, the problem with these studies is that they do not permit unambiguous conclusions because confounding with extraneous components of variation cannot be ruled out. Of particular interest in this context is the confounding effect of genetic variability, where populations may differ in their genetic composition over very short distances (Green et al., 1983). Furthermore, within-population genetic variability may reflect a large fraction of the total genetic variation in a species, and physiological parameters thought to be independent may in fact be genotype dependent (Koehn and Shumway, 1982).

Molluscs have proven to be an excellent model system for the study of many aspects of ecological genetics. Allozyme genotype(s), particularly heterozygosity, have been used as probes for fitness characters associated with genetic variation, including growth rate and mortality (Singh and Zouros, 1978, 1981; Singh, 1982, 1984; Singh and Green, 1984). The question is not whether heterozygosity is associated with fitness features in bivalves, but what the biochemical mechanism is that makes this association possible. This may provide a common mechanism for the maintenance of genetic variability in populations (Soule, 1980) which permits a response to challenges and selection (Franklin, 1980). Attempts to find unique allelomorphs to monitor environmental abuse have not been successful, but may be after further research. It is clear that using genetic analysis as a monitoring tool would pose most, if not all, the challenges discussed earlier for other types of community and population studies.

(c) Shell structure and chemistry

It is the ability to use mollusc shells to 'map' environmental variation over long time periods that is most appealing; this is being validated in two different ways. First, the suspected great longevity of some species has been validated, often with the aid of new technology (e.g. Thompson et al., 1980; Jones et al., 1983). Second, the ability to use fossilized shells as paleo-environmental indicators has been more convincingly argued (Lutz and Jablonski, 1978a,b; Green, 1979b; Jablonski and Lutz, 1980; Green et al., 1984). Growth and mortality rates on the time scale of the monthly, daily and semi-daily tidal cycle can be estimated (Clark, 1968; Evans, 1972; Richardson et al., 1980), and "annual patterns of microgrowth increments have been observed within the shells of the majority of specimens that have been examined and analyzed in detail" (Lutz and Rhoads, 1980). Annual shell rings are being used for several widely distributed species in environmentally sensitive nearshore habitats (*Mytilus edulis*, Lutz, 1976; *Macoma balthica*, Green, 1973, Green et al., 1983; *Mercenaria mercenaria*, Kennish, 1980). Effects of water chemistry and temperature on shell size or form are reported by Davies and Crenshaw (1971), Jones (1981a) and Storr et al. (1982). Macdonald and Thomas (1982) described the effect of oil, and Ekelund et al. (1983) the effect of chemical waste, on shell growth.

Shell chemistry responding to environmental patterns is less well documented. Rosenberg (1980) believes that "the extent to which shell chemistry of bivalves can be used to make environmental . . . interpretation is, at present, unclear". The use of the shells of living or recent bivalves has fewer problems than the use of fossil shells, e.g. the likelihood of diagenetic alteration of shell chemistry (Raup and Stanley, 1971) is much less. Sturesson and Reymont (1971) found that *Macoma balthica* shells from low-salinity locations had higher Sr and lower Mg and Cu concentrations. Sturesson (1976) reported that *Mytilus edulis* exposed to lead incorporated it into their shells; the highest values were in new CaCO_3 and in old periostracum.

Dodd (1965) found increasing Sr concentrations in *Mytilus edulis* shell with increasing temperature, but this was not observed by Lutz (1981). Koide et al. (1982) argue for the use of bivalve shells rather than tissues in monitoring for heavy metals; they found that byssal threads concentrated transuranic elements. Keckes et al. (1968, 1969) reported rapid uptake and retention of Zn^{65} in *Mytilus provincialis* at measurable levels in shell. Lee and Wilson (1969) described a discharge-related variation of the Sr/Ca ratio in the shells of unionids, but their conclusions are questioned by Rosenberg (1980). Imlay's (1982) review paper provides a recent bibliography on freshwater bivalve shells.

Appropriate analytical methods for the analysis of shells are reviewed by various authors in a book by Rhoads and Lutz (1980). Annual or other periodic rings may be seen on the shell surface, but validation by shell sectioning is essential. For chemical analysis, shell layers can often be separated by heating the sectioned shell, then prying layers apart with a scalpel and analyzing them separately (Tevesz and Carter, 1980b). Typical analytical methods involve the use of atomic absorption spectrometry or mass spectrometry. Analysis across the exposed, unseparated, shell layers in a shell section can be carried out by a transect of individual electron microprobe analyses. Unfortunately, the resolution of 'the probe' is often inadequate (Lutz, 1981; Green et al. unpublished).

What then is the state-of-the-art in the use of shell structure and chemistry to monitor environmental variation? First of all, the use of shell chemistry to estimate paleoenvironments over geological time scales is compromised by the problem of diagenetic alteration. Second, the physiological processes involved are complex and diverse, and general models to predict particular responses from particular environmental stresses are not in sight. For example, the processing of heavy metals by bivalves is complex (Keckes et al., 1968, 1969; Smith et al., 1975), and depends on the organically-bound form of the metal that is involved, the species of unionid, and the details of the recent history of exposure. Physiological responses are often extremely non-linear, for example ratios of Sr and Mg to Ca are influenced by salinity at low salinity but not at higher levels (Dodd and Crisp, 1982). The uptake and the resulting concentrations of some metals vary with the organism's growth rate (Kumagai and Saeki, 1981). Third, the very fact that shell structure and chemistry 'maps' the variation of the natural environment (e.g. exposure, temperature, salinity) also means that these influences will always make the use of shells for monitoring environmental 'quality' difficult (if by that one means the influences of human-derived pollutants). Finally, periodic rings or shell layers may not in fact be periodic (Haskin, 1954; Jones, 1981b), although new methods have made this less of an art and more of a science. In summary, it still appears that the use of shell structure and chemistry to monitor the environment has great potential, but there are serious problems which have yet to be overcome. For the present, the best studies of this kind will be those which control potentially confounding variables, such as taxonomic, age, and natural environment variables.

APPROACHES TO BETTER MODELS

Selection and formulation of variables

We have already discussed how bivalve molluscs satisfy the basic requirements of a good response system for monitoring environmental variation. Here we will argue for the exploration of the possibilities for new, sensitive response variables, and give some examples of innovation. A general review is given by the Food and Agriculture Organization (1976) and by Green (1985).

Measurement of physiological and behavioural responses in the field is often difficult, and the result is often high variability in the apparently unimpacted situations (e.g. Nielsen and Nathan, 1975). Bayne et al. (1977) described an ingenious system for measuring the feeding rates and oxygen consumption of bivalves under field conditions, and reported results for *Mytilus edulis* and *Cardium edule*. Brafield and Newell (1961) described the behaviour of *Macoma balthica*, illustrating the kind of methodology which can permit the use of behavioural response as an informative variable, under field conditions. Under controlled laboratory conditions, quite subtle physiological and behavioural responses can be measured precisely. An early example is Hamwi and Haskin's (1969) method for determining respiration and pumping rates in *Mercenaria*. Holopainen (1977) described how to measure respiration in minute *Pisidium*. Gill ciliary beat rate can provide a precise measure of response to environment (Paparo and Sparks, 1977; Sparks et al., 1981). The freezing tolerance of *Mytilus* responding to oil contamination was determined by Aarset and Zachariessen (1982). Gallagher and Mann (1981) described a simple, inexpensive and rapid method for assessing the nutritional status, or "condition", of bivalve larvae. Salanki and Varanka (1976) used a "mussel actograph" to record the rhythms of movement of the valves, influenced by Cu and Pb in their environment. Field observation of *Mytilus edulis* growth rates was described by Lubinsky (1967). A field experiment to determine environmental influences on growth rate in six species of bivalves was described by Gillmor (1982). McCuaig and Green (1983) and Green et al. (1983) derived growth rate parameters from field measurements of shell annual rings, and Watling (1982) evaluated the effects of heavy metals on larval growth of three oyster species in the laboratory. Methods for assessing the effects of environmental stress on reproduction and larval development have been described by Davis and Hidu (1969) and Bayne (1975). What are required now are refined studies which evaluate and compare potential response variables, ranking them by their ability to indicate environmental state and perhaps combining them into indices. Examples are given by Bayne et al. (1976, 1981), Mann (1978), and Ekelund et al. (1983). Indices, in the sense of arbitrary compounds of simple response variables, are not generally advisable (Green, 1979a, 1980). Multivariate descriptions of response are preferred because the components of the data can express themselves, instead of a

preconceived component being forced onto the data. The use of cluster analysis to identify indicator species, or otherwise reduce multivariate response data which include bivalve molluscs, is described by Green and Hobson (1970), Smith and Greene (1976), and Mahoney and Livingston (1982). Kvalheim et al. (1983) used principal component analysis to describe the structure of pollutant concentration data. The derivation of multivariate vectors which maximize the strength of the relationship between a response vector and an environmental predictor variable (canonical analysis, discriminant analysis) is described and illustrated by Green (1972, 1979a).

Predictor variables should include measures of natural environmental variation that may obscure or be confounded by the relationship between an impact and the biological response. The influences of physical factors, such as salinity and temperature, have been discussed. Here we will consider relevant biotic variables, which are seldom incorporated into models. The characteristics of individuals, such as age, size, weight, sex and reproductive condition have effects on the uptake of, and response to, heavy metals by bivalves (Cunningham, 1979). Bayne et al. (1981) showed how sublethal responses to polluting hydrocarbons can be affected by the season, reproductive state and the size of the organism, and how such responses can be statistically adjusted for these variables. Fischer (1983) described the use of shell weight as an independent variable in relation to cadmium content. Freeman and Dickie (1979) showed that genetic differences between stocks of *Mytilus edulis* must be taken into account when the species is used as an environmental indicator. Fisher (1977) found that diatoms from estuarine environments were more tolerant to exotic chemicals than were diatoms of the same species from the open ocean, suggesting a genetically-based pre-adaptation to previously unexperienced impacts, derived from adaptation to a more rigorous natural environment. Parasites can influence behaviour, tolerance, and growth (e.g. Hulscher, 1973). Parasite species and levels of infestation can vary over short distances within a population (Threlfall and Goudie, 1977). Spatial patterns of food availability, which may not be obvious, can determine abundance (Tunncliffe and Risk, 1977) and growth rate (Beukema et al., 1977). Predation can determine size distributions (Goss-Custard et al., 1977) and shell morphology (Vermeij and Covich, 1978; Vermeij and Currey, 1980). Pielou (1975) shows how competition alone can produce species zones on environmental gradients. The applied ecologist should consider these examples, and think about the implications if spatial variation in shell form or growth rate or tolerance was used as a measure of impact when the spatial pattern was partly caused by, or coincided with, patterns in parasite load, genetic composition, or competition pressures which had not been included in the model as explanatory, or predictor, variables.

How should design be improved?

Green (1979a, 1984a, 1985) and Bernstein and Zalinski (1983) discussed the principles of good design in environmental studies. Such classic texts on

sampling and experimental design as those of Cochran (1953) and Winer (1971) should be at the elbow of any environmental biologist. The best biologically-oriented general statistics book, which has a good section on design, is that of Snedecor and Cochran (1980).

We perceive three ways in which design can be improved. First, confounded variables should be identified and controlled. Second, experiments performed in the field, or laboratory experiments coupled to descriptive field studies, should more often be part of our research. Third, descriptive field studies should be conducted over a number of years in order to assess among-year variability in the natural system. Each of these points will now be discussed briefly.

The control of extraneous variables is considered by Gruenberger (1964) to be the most important in his list of 13 attributes of good science. Let us take several examples of confounded variables, and see how we would use the design to control the extraneous variable. Reading (1979) found that tide level-related factors influenced the shell length of *Macoma balthica*; if we were using *M. balthica* growth rate as a response variable in an impact study, we should use only one tide level or use a stratified design containing several tide levels as the strata. Frazier (1976) described the seasonal variation of metal dynamics in oysters; the use of metal concentrations as the response variable in a pollution study should be at one time of year only or stratified by time of year. Theede (1980) found that the sensitivity of estuarine molluscs to Cd depended strongly on salinity; if salinity cannot be controlled then it should be measured routinely to be able to evaluate the lack of balance in the design when analysing and interpreting the data.

Experiments carried out in the field achieve the best of both worlds: (1) the validity of experiment with random assignment of true replicates to treatment levels; and (2) realistic conditions. Mahoney and Livingston (1982) conducted a long-term monitoring study and then evaluated the effect of fish predation on macrobenthos, including bivalves, by carrying out cage experiments with controls. Dickie et al. (1984) assessed the genetic influences on growth in different mussel stocks by conducting transplant experiments. Good examples of studies with complementary field observations and laboratory experimentation are those of Leveque (1971), Smith et al. (1975), Mann (1978), Sparks et al. (1981), and Green et al. (1983).

One-year impact studies are finally receiving the skepticism that they deserve. When they are entirely observational in character, there is no true replication, and sampling variability is typically used as a 'pseudoreplication' (Manly and Wright, 1982; Hurlbert, 1984). Green (1984a, 1985) argues for the use of among-year variability as the error term in such observational studies, but this implies a pre-impact study over sufficient years to provide adequate among-years degrees of freedom. The long-term monitoring study described by Livingston (1982) and Mahoney and Livingston (1982) is a good example, as is the study in Baie de Concarneau reported by Chardy and Glemarec (1977) and Glemarec (1978). For very long records, paleo-ecological studies are valuable. Delorme (1982) uses ostracod species from

cores of Lake Erie sediments to assess the prehistoric record, and concludes that there has been little or no long-term trend in trophic state during that period.

Statistical analysis and modelling

Ideally, models, tests of hypotheses and statistical analyses in general should be based on balanced sampling or experimental designs (preferably some of both), and true replication randomly allocated to treatment levels or to locations subject to different conditions. (In this last case only some kind of artificial substrate or transplant experiment could satisfy the conditions.) Good examples of analyses based on orthogonal (balanced) factorial designs are described by Kastendiek et al. (1981), Macdonald and Thomas (1982) and Theede (1980).

Multivariate descriptions of biological response have been discussed; good designs lead to good statistical analyses and related tests of hypotheses for the multivariate, as well as the univariate, case (see Green, 1979a, 1980). Most ecologists think first of clustering and ordination when they think of multivariate statistics. The top data matrix in Fig. 1 represents the situation where there is no a priori conceptual partitioning of the data, for the purpose of the data analysis. The variables (columns) are all response variables, and the samples (rows) are not grouped a priori (into treatments, locations, times, etc.). We do not have an a priori model to fit to the data so as to

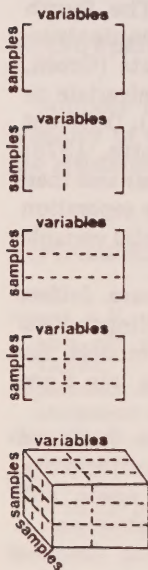


Fig. 1. Symbolic representation of data matrices which give rise to various statistical models. Reproduced from Fig. 3.3 of Green (1979a).

'structure' it; rather we have empirical data whose internal structure we wish to describe. The null hypothesis that there is no structure in the data corre-

sponds to the null hypothesis that all correlations are really zero, except for sampling error. This can be tested using Bartlett's test of sphaericity (Green, 1979a, p. 139; Green et al., 1983), and the description of structure should proceed only if the null hypothesis is rejected. If we expect a structure which will best be described by groupings of the samples and/or the variables, then we do cluster analysis. If we expect a structure best described by continuous gradients, we perform an ordination analysis of some kind (principal components analysis, factor analysis, multidimensional scaling, etc.). These were the first multivariate techniques to be employed in ecology, and they can be used effectively (e.g. Reys, 1976; Mahoney and Livingston, 1982), but ecologists should be aware that every univariate statistical model is only a special case of a general multivariate model. For example, the second symbolic data matrix in Fig. 1 implies an a priori structure of the variables, usually into a predictor set on the right and a response set on the left. If the left-hand set has one column we have simple regression or multiple regression, depending on whether the right-hand set has one, or more than one, column. If the left-hand set has more than one column then there is more than one response variable, and we have canonical correlation analysis (Green, 1972). The third symbolic data matrix in Fig. 1 implies a priori structuring of the samples, into groups, treatments, locations, or whatever. If there is only one column we have analysis of variance, and if there is more than one then we have multivariate ANOVA and its extension, discriminant analysis (Green, 1971; Salmon and Green, 1983). The fourth symbolic data matrix combines a priori variable structure and sample structure, and leads to univariate (Bayne et al., 1981) or multivariate (Green, 1974) analysis of covariance. More complex factorial designs, univariate or multivariate, can be used (bottom symbolic data matrix in Fig. 1). Different analyses can also be combined, for example (Green and Vascotto, 1978) clustering the biological response variable set (species abundances) and then using multivariate ANOVA and discriminant analysis to assess the separation of the 'faunal groups' thus defined, in a multivariate environmental variable space.

The subject of modelling is too vast to treat in any detail here. Jeffers (1982) broadly surveys modelling (see also Green, 1984b), including models not often considered for ecological applications (e.g. matrix models, see Green, 1979b, Green et al., 1984; catastrophe theory models, see Kerr, 1977).

Finally, good display of results is essential, and multivariate statistical methods can lead to excellent visual displays. Symbol coded scatter plots, response surfaces, clusters of samples plotted in ordination space, and many other techniques are possible (Green, 1979a, 1980). Whether one is using multivariate statistical methods to test hypotheses or to describe patterns, good display is necessary because multivariate tests are very power-

ful (especially when there are many variables and many samples) and 'significant' results are likely. Therefore, in the end the problem is the same, to describe effectively what is going on *biologically* in the data.

SUMMARY

In any model of a biological response, the time scale of interest is perhaps the most important consideration. Short-term changes in the behaviour and physiology of a particular species may be useful in sensitive models measuring a particular, subtle environmental impact. Longer term changes in communities (species richness, diversity, composition), population processes of a species (birth and death rates), and the genetic structure of a population may be needed in models of biological response to greater and/or longer lasting changes in the environment.

Bivalve molluscs show promise as components of both long- and short-term models of environmental change. Their relatively well-studied behavioural, physiological, population and community responses to change may, with careful planning, be useful. In addition, all bivalves possess an accretionary shell. This shell enables researchers to model long-term changes in the environment which have affected sensitive behavioural and physiological processes (e.g. metal uptake). Thus, one has the best of both worlds, with the sensitivity of a short-term study in a long-term assessment.

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Acidification effects on shell morphology
and trace metal uptake in the
freshwater clam Elliptio complanata

by

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Abstract

Three independent studies were used to examine the covariation between lake water chemistry and Elliptio complanata (Bivalvia: Unionidae) shell morphology. Clams were collected from 40 central Ontario lakes that were located both on and off the Precambrian Shield. Large, thicker shelled clams were found in more alkaline, less acidic lakes where the processes involved in calcium carbonate shell formation are more favourable. Large, narrower shelled clams were found in more turbulent (more exposed) habitats within lakes. This morphology could be adaptive because it would enhance the clams' ability to maintain its' position in turbulent habitats.

Clams were collected from low and high exposure areas in each of four small Precambrian Shield lakes, of similar size and shape, that varied in acidity. Clams from low exposure sites were smaller and lighter than those from high exposure sites. This could be adaptive because small light shells would be less likely to sink in low exposure soft silt areas while large heavy shells would be less easily dislodged by wave action in high exposure sandy areas. There was no relationship between water chemistry and shell morphology.

Clams were reciprocally transplanted between three small lakes, of similar size and shape, that were positioned on an acidity gradient. One year's growth and final trace metal concentrations (Cu, Cd, Zn, Mn) were evaluated. Growth during that year did not appear to be related to the acidity of the clam's destination. Tissue metal concentrations were a function of both population source and transplant destination.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
1. INTRODUCTION	1
2. METHODS	
2.1 Forty lake comparison of shell form	4
2.2 Within- vs among-lake comparison of shell form	9
2.3 Reciprocal transplant experiment: shell growth	19
2.4 Reciprocal transplant experiment: trace metals	20
3. RESULTS	
3.1 Forty lake comparison of shell form	23
3.2 Within- vs among-lake comparison of shell form	26
3.3 Reciprocal transplant experiment: shell growth	37
3.4 Reciprocal transplant experiment: trace metals	38
4. DISCUSSION	
4.1 Forty lake comparison of shell form	44
4.2 Within- vs among-lake comparison of shell form	45
4.3 Reciprocal transplant experiment: shell growth	46
4.4 Reciprocal transplant experiment: trace metals	47
5. CONCLUSIONS	50
LITERATURE CITED	51

Growth of *Lampsilis radiata* (Bivalvia: Unionidae) in Sand and Mud: A Reciprocal Transplant Experiment

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Hinch, S. G., R. C. Bailey, and R. H. Green. 1986. Growth of *Lampsilis radiata* (Bivalvia: Unionidae) in sand and mud: a reciprocal transplant experiment. Can. J. Fish. Aquat. Sci. 43: 548–552.

To examine the effects of habitat on shell growth and form, freshwater unionid clams (*Lampsilis radiata*) were reciprocally transplanted between a sandy and a muddy site in Inner Long Point Bay, Lake Erie. There were significant differences in the initial shell dimensions of the two populations, with the sand clams being larger and less obese than the mud clams. Pretransplant growth rate analysis, using annual rings, showed that long-term growth in the sand was greater than that in the mud. After 16 wk the transplanted clams were recovered. Overall growth rate was affected by the source of the clams, while transplant destination affected shape change (height growth per unit length growth). This suggests that shell growth rates may be under direct genetic control, while shell shape can be adaptively modified by environmental cues.

Pour examiner les effets de l'habitat sur la croissance et la forme de la coquille, on a transplanté des coquillages unionidés d'eau douce (*Lampsilis radiata*) d'un site sableux à un site boueux et vice versa. L'expérience a eu lieu dans la baie Long Point intérieure du lac Érié. Les dimensions initiales des coquilles étaient très différentes d'une population à l'autre, les coquillages de sable étant plus gros et moins obèses que les coquillages de boue. L'analyse du taux de croissance avant la transplantation, d'après les couches d'accroissement annuel, a montré que la croissance à long terme est plus forte dans le sable que dans la boue. Après 16 sem, on a récupéré les coquillages transplantés. L'origine des coquillages influait sur leur taux de croissance global, alors que le milieu dans lequel ils étaient transplantés conditionnait leur forme (croissance en hauteur par unité de croissance en longueur). Ces résultats portent à croire que le taux de croissance des coquilles est commandé directement par des facteurs génétiques, alors que la forme de la coquille peut subir des modifications adaptatives dictées par les conditions environnementales.

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Many researchers (Isely 1911; Wilson and Clark 1914; Grier 1920; Eagar 1948; Agrell 1949; Negrus 1966; Harman 1972; Green 1972; Kat 1982) have observed correlations between habitat and the growth (inferred from size) and form of unionid clams. However, field experiments are necessary to verify such observational data. Biological monitoring using unionids is receiving increasing attention (Foster and Bates 1978; Curry 1977–78; Forester 1980; McCuaig and Green 1983; Mitchell 1984). A better understanding of the factors affecting the growth and form of unionids will help to assess them as biological monitors of freshwater environments.

On the basis of a field experiment, Kat (1982) determined that growth rates of the freshwater unionid *Elliptio complanata* were reduced on muddy substrates. He hypothesized that on mud substrates clogged filtration systems would cause a reduction in feeding efficiency. Conversely, Newell and Hidu (1982), using a laboratory transplant study, noted that the marine bivalve *Mya arenaria* grew more rapidly in mud than in sand. Their explanation was that food availability was greater in muddy sediments. Reciprocal transplants in the field have recently been used to examine the relative importance of habitat type and genotype on the growth rate of the marine bivalve *Mytilus edulis* (Widdows et al. 1984; Dickie et al. 1984). The purpose of this study was to utilize a reciprocal transplant experiment to show how habitat type affects growth rate and

and shell form in the freshwater unionid *Lampsilis radiata* (Gmelin 1792).

Materials and Methods

Two sites in Inner Long Point Bay, Lake Erie (42°38'N, 80°24'W), were chosen for the study on the basis of substrate type, as reported in Heathcote (1981). One site, located south of St. Williams, Ont. had a muddy bottom whereas the other, located south of Turkey Point, Ont., had sand (Fig. 1). Selected environmental parameters were measured at both sites (Table 1). R. C. Bailey (unpubl. data) sampled 50 sites in the Inner Bay and found that the two sites in this study fell at nearly opposite ends of a sand–mud gradient.

On June 4, 1984, 183 *L. radiata* were collected from the two sites using SCUBA. Clams were aged by counting annual rings. A letter and number code were scribed onto each shell to identify collection site and individuals. The initial length, height, and width of each individual (as defined in Tevesz and Carter 1980, our “width” measurement is equivalent to their “thickness”) were measured using vernier calipers to the nearest 0.01 mm. Half of the clams were randomly chosen and replaced as controls at each site, while the remaining half were taken to the other site. The clams were “replanted” by hand in their normal living position (i.e. anterior end in the sediment). On September 21, 1984, after 16 wk of growth, marked indi-

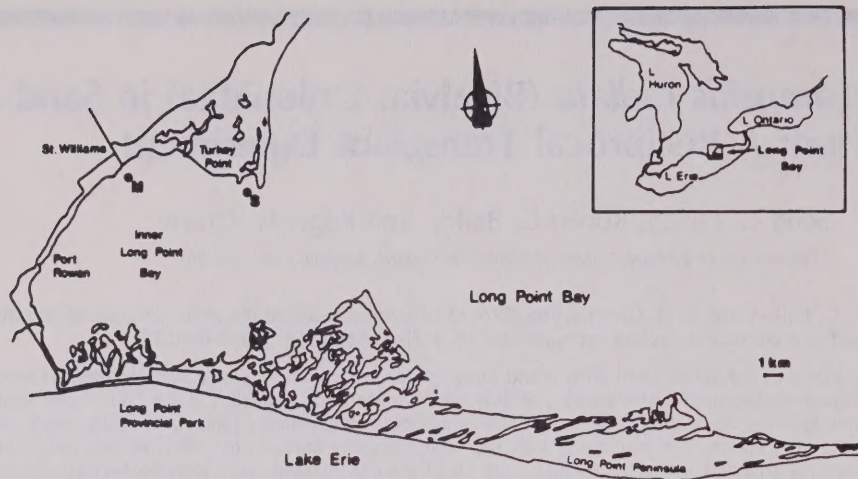


FIG. 1. Inner Long Point Bay showing the mud (M) and sand (S) transplant sites. Inset shows location of bay in Lake Erie.

TABLE 1. Means (SD) of environmental measurements from the mud and sand transplant sites. Measurements taken on June 21, 1984.

	Mud site (n = 3)	Sand site (n = 3)
Depth (m)	2.50 (0.06)	1.90 (0.06)
pH	8.44 (0.02)	8.44 (0.04)
Alkalinity ($\text{mg} \cdot \text{L}^{-1}$)	124.33 (0.29)	107.33 (0.76)
Calcium ($\text{mg} \cdot \text{L}^{-1}$)	44.10 (2.44)	37.01 (1.41)
% loss on ignition	2.36 (0.20)	0.64 (0.34)
% sediment sample that did not pass through 75- μm sieve	20.03 (6.12)	82.47 (2.31)
Macrophyte species present	<i>Vallisneria americana</i> <i>Najas</i> sp. <i>Myriophyllum</i> sp. <i>Potamogeton praelongus</i>	<i>Chara</i> sp.

viduals were recovered from both sites. Final lengths, heights, and widths were remeasured.

The means of the initial log-transformed shell length, height, and width measurements at each site were compared using multivariate analysis of variance (MANOVA). This was followed by calculation of the normalized discriminant vector describing the difference in the mud and sand populations with respect to shell dimensions.

Long-term growth was assessed using analysis of covariance (ANCOVA), which compared growth ring data from the mud and sand populations using the Walford plot model (McCuaig and Green 1983). This model assumes von Bertalanffy growth curves. Growth ring data were collected by measuring lengths of the most legible pair of consecutive annual rings on each clam. This sampling strategy differs from that of McCuaig and Green (1983) and Green et al. (1983) who measured as many pairs of consecutive annual rings on a single clam as possible.

Short-term growth was assessed from the reciprocal transplant experiment data. A factorial multivariate analysis of covariance (MANCOVA) was carried out using final length, height, and width of the clams as the dependent variables and initial length, height, and width as the covariates. This is a multivariate factorial analogue of the Walford Plot ANCOVA utilized and described by McCuaig and Green (1983). It en-

ables one to determine both the statistical significance and the nature of differences in growth along the three dimensions due to the source of the clams, their transplant destination, and the interaction of these two factors.

A factorial ANCOVA was also run on each dimension (length, height, and width) separately as an aid to showing how growth in each dimension was affected by source and destination. In each of these analyses the final size of the dimension was the dependent variable and the initial size was the covariate.

Results

The initial dimensions of the clams (Table 2) were significantly different (based on Wilks criterion, MANOVA $F(3,104) = 130.14$, $P < 0.01$). The normalized discriminant vector derived from this analysis (Table 2) indicated that the clams from the sand site were longer and taller but less obese (narrower at a given length) than the clams from the mud. Relative height (height per unit length) was less in the clams from the sand. If the difference in the populations was only a matter of size, then all of the discriminant coefficients would be equal. This is based on an allometric growth model described by Mosimann (1970). The fact that the width coeffi-

TABLE 2. Means (SD) of initial shell dimensions (mm) in the total sample and in each group, and the normalized discriminant coefficients from a comparison of the sand and mud clams.

	Total (n = 107)	Sand (n = 53)	Mud (n = 54)	Normalized discriminant coefficient
Length	58.7 (10.6)	68.0 (5.7)	49.6 (4.3)	0.78
Height	35.2 (6.0)	40.6 (2.6)	29.9 (2.9)	0.58
Width	25.7 (6.0)	30.9 (3.4)	20.7 (2.7)	0.26

TABLE 3. Normalized discriminant coefficients and probability levels for factorial MANCOVA of length, height, and width growth of transplanted clams.

	Length	Height	Width	P
Source	0.429	0.895	0.142	0.023
Destination	-0.593	0.803	0.049	0.008
Source × destination	0.490	0.867	0.096	0.284

TABLE 4. Probability levels for factorial ANCOVA's of length, height, and width growth of the transplanted clams (df = 1,103).

	Length	Height	Width
Source	0.096	0.004	0.365
Destination	0.121	0.018	0.786
Source × destination	0.202	0.095	0.781

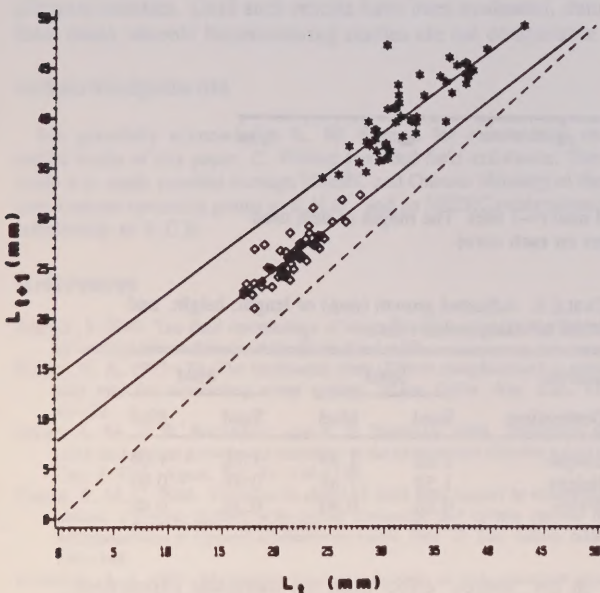


FIG. 2. Walford Plots of the lengths at consecutive annual rings for *L. radiata* from the sand (*) and mud (□) sites. Length at time t is L_t ; length at time $t + 1$ is L_{t+1} .

cient is much smaller than the length coefficient indicates a proportionately smaller increase in width, relative to increase in length, for the clams from the sand.

The mean ages of clams collected from the sand and mud populations were 11.1 ± 1.7 ($\pm 95\%$ confidence limits) and 10.4 ± 1.7 yr, respectively. These means are not significantly different (t -test, $P > 0.05$); therefore, differences in initial shell dimensions are not a result of differences in population age.

Clams from the mud and sand populations have the same relative growth rates (i.e. they approach their asymptotic size at the same age) based on the consecutive annual growth ring data (ANCOVA; Fig. 2). However, the clams from the sand site grow to a greater maximum size (Fig. 3) ($F(1,100) = 290.5$, $P < 0.01$) and thus have a greater absolute growth rate. The growth equations derived from the annual ring data are as follows:

Mud population:

$$L_t = 67.7(1 - e^{-0.111t})$$

Sand population:

$$L_t = 115.3(1 - e^{-0.111t})$$

where L_t = length at time t and t = time in years.

We recovered 58% of all marked individuals: 64% from the sand site and 54% from the mud. The factorial MANCOVA of the pre- and post-transplant data (Table 3) showed that both the source of the clams and their destination had significant effects on their shell growth in the reciprocal transplant experiment. There was no interaction between source and destination. The discriminant coefficients indicate that the "source" effect is on overall growth rate (i.e. all coefficients are positive). Clams from the sand site grew faster than those from the mud site. Destination affected growth in height relative to growth in length (i.e. the length and height coefficients have opposite signs). Both control and transplanted clams placed at the mud site grew more in relative height (height growth per unit length growth) than control and transplanted clams placed at the sand site.

The ANCOVA's of each dimension (Table 4) show similar results. The adjusted growth of each group of clams along each of the three dimensions was determined from these ANCOVA's (Table 5). To obtain these, the mean final size of each dimension for each group was adjusted by the ANCOVA for differences among the clams in initial size, and then subtracted from the initial size of the dimension.

Discussion

We determined from initial shell sizes that clams from the sand are longer, taller, and less obese than those from mud. Analysis of annual growth rings shows that clams from the sand grow faster than those from mud although both groups reach their asymptotic size at the same age. The results from the transplant experiment also indicate that clams from the sand grow faster than those from mud. These three independent analyses all result in a similar conclusion: *L. radiata* from sand grow faster and are less obese than those from mud.

Clams from the sand habitat had greater lengths and heights than those from the mud. Kat (1982) speculated that a similar trend observed in the lengths of *E. complanata* from muddy substrates resulted from a reduction in feeding efficiency caused by fine sediments clogging the clams' filtration system. Stansbery (1970), however, suggested that muddy substrates reflect reduced water flow regimes, and therefore a reduction in available food may be a cause of reduced unionid growth in muddy habitats.

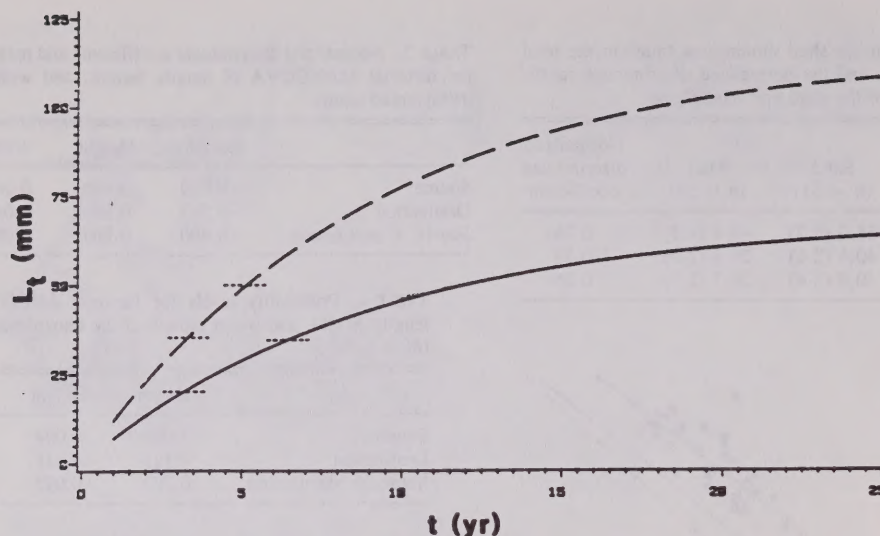


FIG. 3. Growth curves for *L. radiata* from the sand (---) and mud (—) sites. The ranges of data used to derive these curves are indicated by horizontal broken lines on each curve.

Both the clogged filtration system and the nutritive-stress hypotheses might have been used to explain variability in *L. radiata* growth rates. However, they can not explain greater shell obesity in the clams from muddy sediments. Variation in shell dimensions may represent morphological adaptations to the environment. Large and long shells will assist the clam in maintaining its position in and moving through sandy, more turbulent areas. Smaller, more obese shells will permit the clam to present a broad surface in nonturbulent, soft-silt areas thereby enabling the clam to maintain its position by reducing the amount of sinking.

Horn and Porter (1981) noted that in Lake Waccamaw, obesity of *Lampsilis* sp. was positively correlated with percent organic matter of the sediment. Tevesz and McCall (1979), after observing a similar phenomenon in *L. radiata siliquioidea* in western Lake Erie, suggested that this characteristic enhances buoyancy and helps preclude sinking in soft sediments. The result from the present study agree with those of Tevesz and McCall.

Adaptations of a species to different habitats can be environmentally induced (phenotypic plasticity) or directly inherited characteristics. Results from the reciprocal transplant experiment suggest that both phenomena are occurring in *L. radiata*. The source of the transplants was most important in predicting variation in overall shell growth, indicating a genotypic adaptation of shell growth rate to the clam's original environment. Variability in relative height growth, however, was a function of the clam's destination. Clams that were left in the mud tended to grow relatively taller regardless of their source. A similar response in shell form was observed by Newell and Hidu (1982) for transplanted *M. arenaria*. They suggested that greater relative shell heights in transplants to mud is an adaptive characteristic that would reduce clam sinkage in soft substrates. Although greater relative width growth (increase in obesity) might have been predicted in clams that were transplanted to the mud, there is so little growth in width in adult clams over one season that such differences were hard to detect.

The argument for a genetically controlled growth rate is

TABLE 5. Adjusted growth (mm) of length, height, and width of transplanted clams.

Source:	Sand		Mud	
Destination:	Sand	Mud	Sand	Mud
Length	1.82	1.39	1.12	1.08
Height	1.58	1.68	0.41	0.94
Width	0.80	0.80	0.36	0.48

based on the "source" effect from the transplant experiment. Environmental effects on gene expression could have occurred early in the lives of these clams, and thus the "source" effect may not necessarily represent genetic differences between the two populations. However, preliminary electrophoretic data (R. C. Bailey, unpubl. data) suggest differences in allelic frequencies between mud- and sand-dwelling *L. radiata* sampled from Inner Long Point Bay.

Differential selection pressures may be present in the different habitats. Thus, genetic differences in growth rates could arise between the mud and sand populations of *L. radiata*. These differences may be maintained through reduced gene flow between the populations as a result of habitat preferences among fish hosts of the glochidia (parasitic larval clams). Alternatively the transplants may have been exhibiting "phenotypic latency." The potential for phenotypic responses in growth rates of the transplants to their new environment may have existed, but the length of exposure was not sufficient for these responses to be elicited.

Any adaptation would be expected to optimize morphological and physiological responses of a population to its environment (Gartner-Kepkay et al. 1980). This phenomenon has important implications for unionid biomonitoring studies. These studies usually entail taking clams from a relatively "clean" source and transplanting them to areas where trace contaminant levels are to be determined. If investigators do not consider the fact that genetically different populations of a monitor species can exist, comparisons of studies over time

could produce misleading results, since contaminant uptake may be related to genetically determined growth rates. For instance, a study measuring uptake of a contaminant using clams taken from a sandy habitat may not be comparable with another year's study using the same species of unionid taken from a silty habitat. Standardizing the source and genotype of the unionid biomonitors could make results of these studies more comparable. This may be possible through laboratory culturing of the clams, which is now possible without fish hosts (Isom and Hudson 1982; Hudson and Isom 1984).

Research is needed to determine the effects of differential trace contaminant uptake in populations of unionids taken from different habitats. Until such results have been evaluated, data from many unionid biomonitoring studies are not comparable.

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Within- and among-lake variation in shell morphology
of the freshwater clam Elliptio complanata

(Bivalvia: Unionidae) *from south-central Ontario
Lakes.*

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Key Words: shell morphology, exposure, alkalinity, Elliptio
complanata, Unionidae

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Abstract

To compare the relative magnitude of variation in shell morphology within and among lakes, E. complanata were collected from low and high exposure areas in each of four small lakes in south-central Ontario. Nested ANOVA's on shell length, height, width and weight revealed that shell morphology varied much more between sites of differing exposure within a lake than among lakes of differing alkalinity. Canonical variate analysis showed that clams from high exposure areas had larger and proportionately taller and heavier shells than those from low exposure areas. There was no relationship between alkalinity of lakes and shell morphology. These results suggest that the use of unionid shell morphology to predict ^{long-term} whole lake water chemistry (e.g. alkalinity) requires sampling designs which take into account within-lake variation in shell morphology.

Introduction

Morphological variation in freshwater unionid clams is correlated with variation in turbulence or exposure in the benthic environment. Unionids which occupy exposed habitats in lakes are smaller and more obese (wider at a given length) than conspecifics which occupy less exposed habitats (Coker et al. 1921; Brown et al. 1938; van der Schalie and van der Schalie 1950; Stansbery 1961; Green 1972; Clarke 1973; Tevesz and Carter 1980). This may be a stunting effect of wave action as clams would ~~require~~ require a continuous energy expenditure just to stay in place (Brown et al. 1938). Alternatively individuals with small obese shells may be less often dislodged by turbulence and shifting currents that occur in lakes during storms (Clarke 1973). In rivers, on the other hand, unionids from areas of fast water flow tend to be large and narrow relative to clams from areas of weak currents (Wilson and Clark 1914; Eager 1978). Wilson and Clark (1914) suggested that these large and narrow shells are adapted for furrowing through coarse substrates and withstanding strong currents. Eager (1978) felt that small obese shells would enable clams to maintain their position in soft silt (i.e. weak current) areas.

Thus, "lake" and "river" unionids show opposite correlations between shell form and exposure or water turbulence, and both adaptive and nonadaptive hypotheses have been proposed to explain this phenomenon. In rivers, size decreases and obesity increases with decreasing exposure (slow current flow) while in lakes, size decreases and obesity increases with increasing exposure. However, Hinch et al. (1986) used a reciprocal transplant experiment to show that growth rates of Lampsilis radiata (Bivalvia: Unionidae) were lower in a mucky, less exposed site than they were in a sandy, more exposed site

in Inner Long Point Bay, Lake Erie. They postulated that the same environmental conditions affecting clams from strong and weak current areas in rivers caused growth variation in clams from the high and low exposure areas in the Inner Bay. However, at very high exposure sites in large lakes (e.g. Brown et al. 1938), suboptimal conditions may reduce growth rates. Thus in large lakes, growth rates may be maximal at an intermediate level of exposure.

We collected similarly aged Elliptio complanata from four relatively small lakes in south-central Ontario. In each lake clams were sampled from a low and high exposure area. We predicted that within a lake, clams from the more exposed site would be larger and narrower than those from the less exposed site. To help assess the importance of within-lake variation in shell morphology due to exposure, we compared within-lake variation to among-lake variation due to alkalinity. It has been suggested that variation in alkalinity can cause variation in shell shape (Mackie and Flippance 1983) and growth rate (Green 1980; Singer 1981).

The four study lakes are located in south-central Ontario on the Precambrian Shield (Fig. 1^{and}2). Although morphologically similar, the lakes vary substantially in alkalinity (Table 1).

The mean wind direction for the ice free season (May to November inclusive) based on monthly wind data summaries from 1983 and 1984 was from the west-south-west (approximately 250°; Environment Canada: Muskoka airfield, 30 km west of Dorset Ont.). We collected 25 clams using SCUBA from a low (southwest) and high (northeast) exposure site (population depths ranged from 1.5-2.5 m) in each lake. Stretches of shoreline that were near inflows and outflows were not considered. To eliminate age structure as a confounding factor only old individuals (greater than 12²⁸ years of age based on annual rings) were collected. These clams are at or are very near their asymptotic size.

The meat was removed from each clam and the shells were allowed to dry. Shell length, height and width (as defined in Hinch et al. 1986) were measured using Mitutoyo 500-110 Digimatic Calipers to the nearest 0.01 mm. Each shell was weighed on a Mettler PB3000 balance to the nearest 0.01 g.

Log-transformed length, height, width and weight were used in the statistical analyses to linearize allometric relationships among the variables. A nested ANOVA estimated the proportion of the total variance of each variable due to variation among the four lakes, between exposure levels within the lakes and among individual clams within the sites. We used SAS PROC NESTED (SAS Institute 1982) to estimate these components of variance.

The relationship between exposure and shell form was described using a canonical variate analysis (Reyment et al. 1984) of the

morphometric variables. Among-lake variation was excluded from this analysis by adjusting the data from each lake to a mean of zero. We did a similar analysis of among-lake variation in morphology by adjusting the data from each exposure site within a lake to the lake mean. SAS PROC CANDISC (SAS Institute 1982) was used to perform the canonical variate analysis.

Confidence ellipses (95%) of the means of the first canonical scores from the "exposure" and "lake" analyses were plotted using Altman's (1978) algorithm programmed in SAS.

Results

In each lake, clams from the high exposure site had longer, taller, wider and heavier shells than those from the low exposure site (Table 2). Components of variance computed from the nested ANOVAs revealed that much more of the variation in shell morphology resulted from differences between exposures (within-lake) than from variation among lakes (Fig. 3). There was a significant ($P < 0.01$, d.f.=4,192) between-exposure effect and a non-significant ($P > 0.20$, d.f.=3,4) among-lake effect, on each variable. The significance levels are not a fair comparison of "exposure" and "lake" effect (the between exposure test has many more error degrees of freedom). However, Fig. 3 is a fair comparison of the relative magnitude of the components of variation.

Canonical variate analysis of between-exposure variation (Table 3) showed that clams from high exposure sites were larger and proportionately taller and heavier than those from low exposure sites. A similar analysis of among-lake variation (Table 4) showed a gradient of decreasing obesity (height and length increasing relative to width) and decreasing shell thickness (height and length increasing relative to weight). The 95% confidence ellipses of the "exposure" and "lake" canonical scores for each site (Fig. 4) show that clams from high exposure sites in all four lakes fell higher on the "exposure" axis than those from low exposure sites. Among-lake variation was primarily due to Bark Lake clams. Bark Lake was intermediate in alkalinity (see Table 1). Differences in Tock and Green Lake clams (lowest and highest in alkalinity respectively) were not important in the "lake" canonical axis. Among-lake variation in shell morphology does not appear to be correlated with variation in alkalinity, but we realize that we have limited power in detecting such a relationship with only four lakes.

Exposure affected variation in unionid shell morphology similarly in all four lakes. Clams from low exposure sites were smaller and lighter than those from high exposure sites. These characteristics may be adaptive because small, light shells would preclude sinking in low exposure, soft silt areas while large, heavy shells would prevent dislodgement by wave action in high exposure, sandy areas. The results agree with those of Hinch et al. (1986). Although other studies have not reported similar results, an analysis of unpublished data from Green (1980) revealed that Anodonta grandis (Unionidae) from the more exposed east shore of Shell Lake ^{North West Territory} N.W.T. were larger than those from the west shore. Thus, the relationship between exposure and growth rate has been shown in three unionid species.

Some researchers have suggested that variation in alkalinity can cause variation in unionid growth patterns. Singer (1981) collected A. grandis from lakes that ranged in pH from 6.6 to 8.6 and noted that clams from the more acidic lakes had slower growth rates. Mackie and Flippance (1983) sampled 53 freshwater habitats along an alkalinity gradient and observed that the weight to length ratio of E. complanata decreased with decreasing calcium content of the water relative to alkalinity, total hardness and pH. Green (1980) sampled one area in Boot and Shell Lakes in the North West Territories for A. grandis and suggested that the larger heavier shells from Boot Lake were a result of the lake's higher alkalinity. However, interpretation of these studies is difficult because within-lake variation in growth rates was not considered. In the present study, among-lake variation was not related to alkalinity after the effects of within-lake variation due

to exposure had been removed. Among-lake variation primarily resulted from differences in the Bark Lake clams, which were thicker-shelled and smaller than clams from the other three lakes. The available lake data do not suggest why ^{the} ~~this~~ difference exists.

Estimates of variability in unionid shell morphology have often been made by sampling only one area of a lake (e.g. Mackie and Flippance 1983; Ghent et al. 1978). Our results indicate that within-lake variation must first be assessed before among-lake variation is examined. This ~~is~~ ^{usually} particularly ^{apply to} ~~true in~~ lakes where wind-induced exposure varies considerably along the shoreline.

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Table 1. Selected morphometric characteristics (data provided by the Ontario Ministry of Natural Resources) and total inflection point alkalinity values (data provided by the Ontario Ministry of the Environment) of the study lakes. Alkalinity values are averages of samples collected from 1978 to 1984.

Lake characteristic	Tock Lake	Bark Lake	Beech Lake	Green Lake
Total inflection point alkalinity ($\mu\text{eq}\cdot\text{l}^{-1}$)	22	153	238	243
Area (km^2)	1.2	1.7	1.4	1.2
Perimeter (km)	7.2	9.0	6.6	5.1
Volume ($\text{km}^3\cdot 10^{-3}$)	6.9	7.7	14.1	6.0
Mean depth (m)	5.9	4.6	10.4	5.0
Maximum fetch (km)	2.5	2.0	2.2	2.0

Table 2. Means (SD) of shell characters from low and high exposure sites in the study lakes. In all groups n=25.

Lake	Length (mm)	Height (mm)	Width (mm)	Weight (g)
Tock low	66.0 (5.4)	33.2 (2.7)	17.7 (1.9)	9.6 (2.9)
Tock high	74.1 (5.8)	37.2 (3.0)	19.6 (2.1)	14.1 (3.6)
Bark low	59.4 (4.5)	28.6 (1.8)	17.8 (1.5)	8.1 (2.0)
Bark high	64.2 (3.1)	30.9 (1.5)	19.4 (1.2)	10.8 (2.1)
Beech low	63.3 (6.3)	31.6 (3.5)	16.7 (2.4)	8.5 (2.8)
Beech high	72.1 (6.3)	36.6 (3.1)	19.7 (2.2)	13.6 (3.7)
Green low	63.0 (8.6)	31.1 (4.2)	16.2 (2.6)	8.8 (4.5)
Green high	66.9 (7.7)	33.1 (3.8)	17.7 (2.5)	11.0 (4.7)

Table 3. Standardized canonical coefficients for each variable and the correlation of each variable with the canonical score from the canonical variate analysis of between-exposure variation.

Shell character	Standardized coefficient	Structure coefficient
Length	-0.13	0.93
Height	0.61	0.96
Width	-0.07	0.88
Weight	0.76	0.98

Table 4. Standardized canonical coefficients for each variable and the correlation of each variable with the canonical score from the canonical variate analysis of among-lake variation.

Shell character	Standardized coefficient	Structure coefficient
Length	0.43	0.50
Height	1.93	0.65
Width	-1.37	-0.14
Weight	-0.50	0.19

List of Figures

- Fig.1. General location of study area in south-central Ontario and detailed location of lakes in relation to provincial highways and towns.
- Fig.2. Four study lakes with the low (L) and high (H) exposure sites in each lake.
- Fig.3. Proportions of total variance in shell height, length, weight and width due to variation among the four lakes, between exposure levels within the lakes and among individual clams within the sites.
- Fig.4. Confidence ellipses of the first canonical scores from the "exposure" and "lake" canonical variate analyses. The four lakes are represented by Tock-To, Beech-Be, Bark-Ba, Green-Gr while L and H represent low and high exposure sites within each lake.



Fig 1

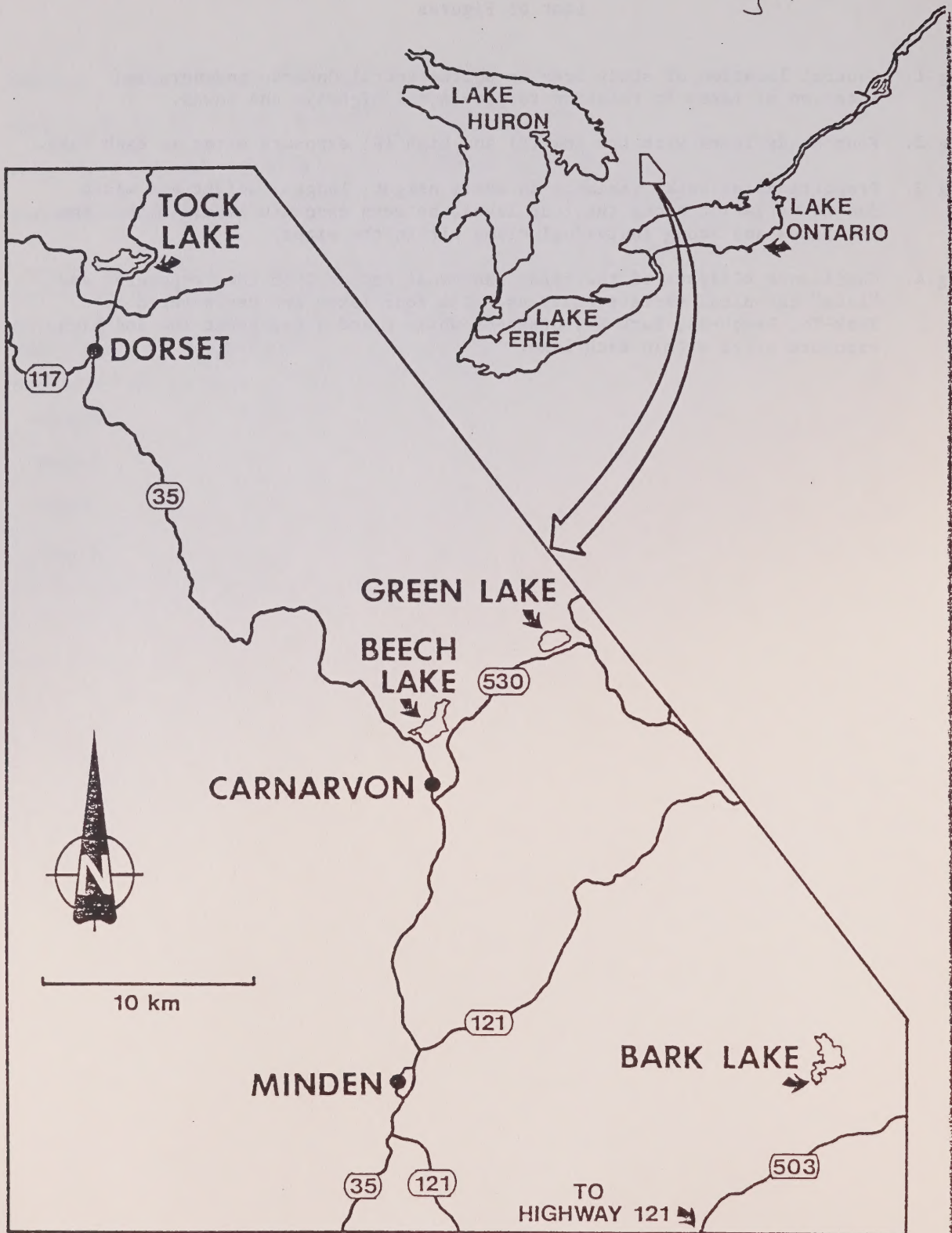


Fig 2

TOCK LAKE



GREEN LAKE



BEECH LAKE



BARK LAKE

2 km



Fig 3

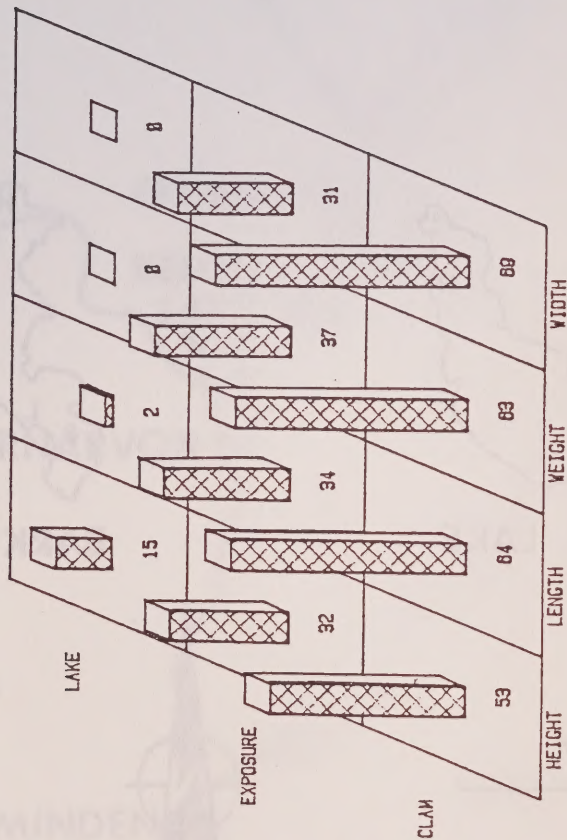
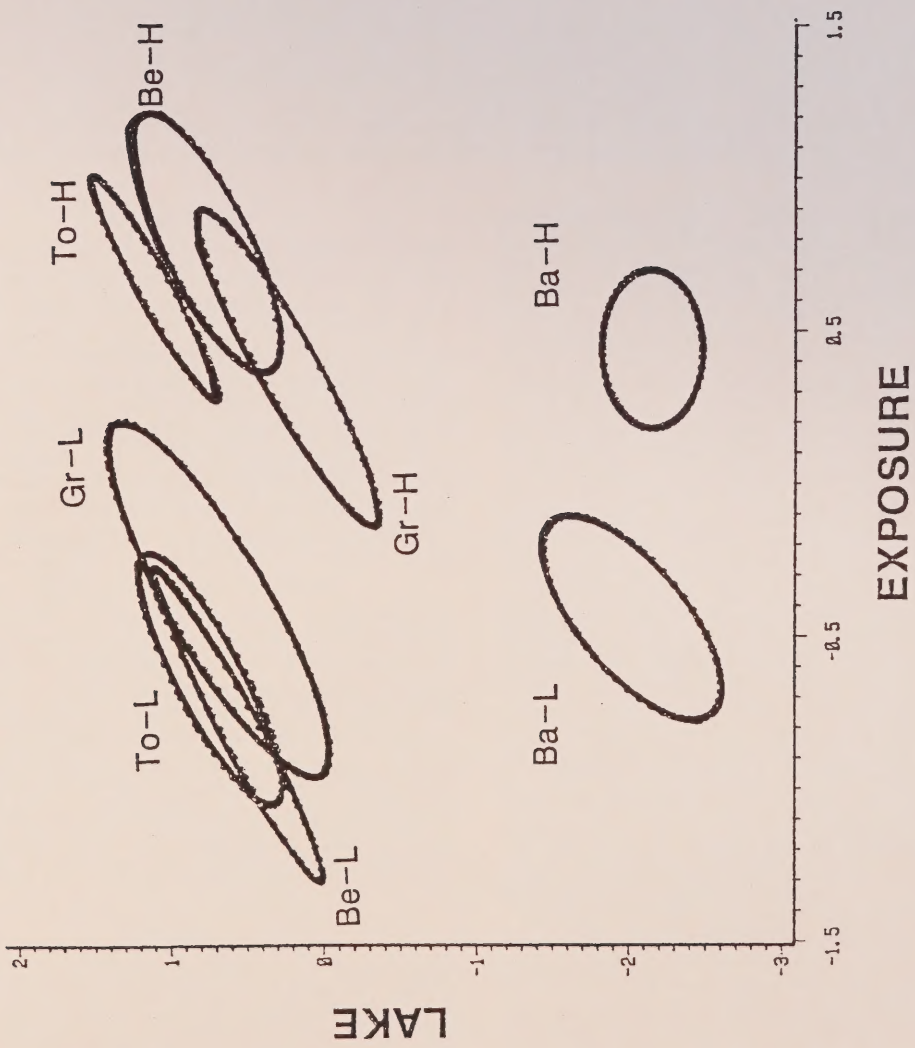


Fig 4



Size- and age-specific patterns of trace metal concentrations in freshwater clams from an acid-sensitive and a circumneutral lake

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We collected freshwater clams (*Elliptio complanata*) from an acid-sensitive and a circumneutral lake in south central Ontario and compared tissue metal concentrations. Clams from the acid-sensitive lake had higher concentrations of Cu and Cd and lower concentrations of Zn and Mn than clams from the circumneutral lake. Tissue concentrations did not reflect metal levels in the water. Competition may be occurring between metals for binding substrate in clam tissue. Clam size and (or) age successfully predicted tissue metal concentrations, but in a metal-specific and tissue-specific manner. Clam biomonitoring studies should therefore control for size and age variability. Lake buffering capability was not very important in influencing size- and age-specific patterns of tissue metal concentrations. However, this conclusion is based solely on data from two lakes.

HINCH, S. G., et STEPHENSON, L. A. 1987. Size- and age-specific patterns of trace metal concentrations in freshwater clams from an acid-sensitive and a circumneutral lake. *Can. J. Zool.* **65**: 2436–2442.

Nous avons récolté des moules d'eau douce (*Elliptio complanata*) dans un lac peu tamponné et un lac presque neutre du centre sud de l'Ontario et avons évalué les concentrations de métaux dans leurs tissus. Les moules du lac acide avaient des concentrations de Cu et de Cd plus élevées et des concentrations de Zn et de Mn plus faibles que les moules du lac neutre. Les concentrations dans les tissus ne reflétaient pas les concentrations des métaux contenues dans l'eau. Il semble y avoir compétition entre les métaux pour se lier aux substrats dans les tissus. La taille des moules ou leur âge, ou la combinaison des deux facteurs, permettent de prédire assez bien les concentrations de métaux dans les tissus, mais d'une façon spécifique à chaque métal et spécifique à chaque tissu. Il est donc essentiel de contrôler la variabilité de la taille et celle de l'âge dans ce genre d'études. La capacité-tampon du lac ne semble avoir que peu d'influence sur les variations des concentrations tissulaires de métaux en fonction de la taille et en fonction de l'âge. Cependant, cette conclusion n'est basée que sur les données obtenues en deux lacs.

[Traduit par la revue]

Introduction

Freshwater unionid clams are used to biologically monitor variation in environmental levels of trace metals through *in situ* (Forester 1980; Imlay 1982; Great Lakes Institute 1984) and transplantation (Krauss *et al.* 1981; Great Lakes Institute 1985) studies. Much of the variability in trace metal tissue concentrations in marine clams has been attributed to variability in clam size or age (Hugget *et al.* 1973; Boyden 1974, 1977; Ayling 1974; Watling and Watling 1976; Bryan and Uysal 1978; Romeril 1979; Cossa *et al.* 1980; Strong and Luoma 1981; Popham and D'Auria 1983; Szefer and Szefer 1985; Cain and Luoma 1986). However, it is uncertain whether freshwater clams exhibit similar patterns. In this study we examine size- and age-specific patterns of trace metal concentrations in the freshwater clam *Elliptio complanata* (Unionidae).

The bioavailability of many trace metals is enhanced by increasing environmental acidity (Tri-Academy Committee on Acid Deposition 1985; Campbell and Stokes 1985; Stokes *et al.* 1985; Kelso *et al.* 1986). In acid-stressed, poorly buffered waters, bioaccumulation of trace metals has been reported in algae (Stokes *et al.* 1985), fish (Scheider *et al.* 1979; Harvey and Frazer 1982; Kelso and Gunn 1984), and clams (Forester 1980; Graney *et al.* 1984). We collected *E. complanata* from an acid-sensitive and a circumneutral lake to compare differences in metal concentrations in clam tissue between the two lakes with differences in size- and age-specific trace metal patterns.

Methods

We collected 50 *E. complanata*, including as wide a size range as possible (lengths, 45.9 to 84.9 mm), using SCUBA from a low exposure (low water turbulence) site in each of two small lakes during the 1st week of August 1985. Beech Lake (45°05' N, 78°42' W) and Tock Lake (45°16' N, 78°53' W) are located in south central Ontario on the

Precambrian Shield (Fig. 1). Although morphologically similar, the lakes differ greatly in total inflection point alkalinities (Tock and Beech lakes, 22 and 238 $\mu\text{equiv}\cdot\text{L}^{-1}$, respectively; Ontario Ministry of Environment data, 1980–1983). Total concentrations of trace metal in water from the two lakes are very similar, although Beech Lake has slightly higher Mn levels (Table 1). Shell length, height, and width (as defined in Hinch *et al.* 1986) were measured to the nearest 0.01 mm using Mitutoyo 500–110 digimatic calipers. Live clams were brought back to the laboratory and were kept alive for 1 week in their original lake water in which time they purged the contents of their digestive system. Clams were then frozen alive in their shells and kept frozen (–20°C) until they were needed for analysis. Clam soft parts were removed from the shell while still frozen using a clean stainless steel scalpel and were thoroughly rinsed with distilled water. The gills were dissected from the remaining visceral mass (body) and analyzed for metals separately because they often contain larger concentrations of metals (Hemelraad *et al.* 1986). Gill water (i.e., water contained in gill tubes) was included in analyses. Tissues were oven-dried at 80°C in preweighed, acid-washed Coors evaporating dishes, and then digested in concentrated ultrapure nitric acid for 18 h. Solutions were heated at temperatures just below boiling until near dryness. After cooling, 1 N ultrapure nitric acid was added to the solutions, which were then filtered through acid-washed ashless No. 40 filter paper. The filtrate was analysed for levels of Cu, Cd, Zn, and Mn using flame spectrophotometry on a Perkin-Elmer model 5000 atomic absorption spectrophotometer. Background correction was used. Blanks were run at the beginning of each analysis session to ensure that extraneous metals were not entering the samples. Standard additions of randomly chosen samples demonstrated that there were no spectral interferences or signal suppression during the analyses.

Dry shells were coated with epoxy resin and a radial cut was made on all left valves from the umbo to the farthest point on the shell ventral margin using a Buehler Isomet low-speed saw with a diamond wafering blade. The cut shell edge was polished with fine-grain diamond grit paper and was affixed to a frosted microscope slide using epoxy resin. Once dried, most of the shell was cut off the slide using the Isomet saw, leaving a thin section of shell, approximately 0.5 mm thick, on the

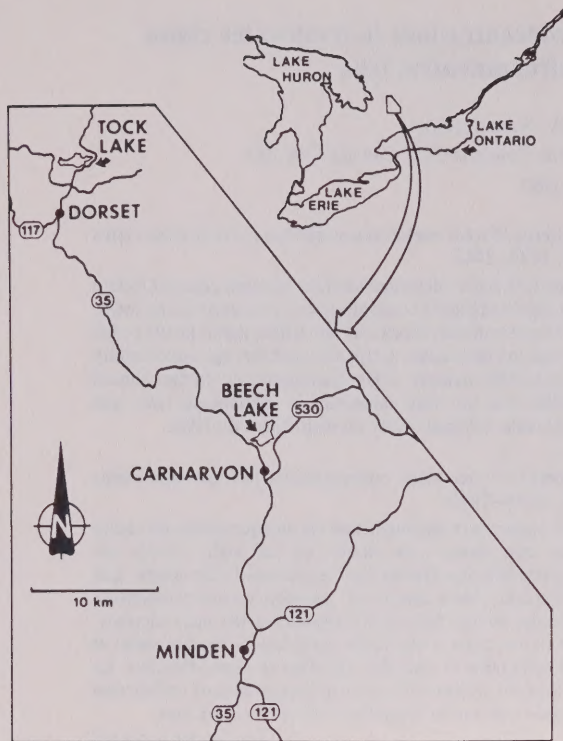


FIG. 1. Location of study areas in Haliburton County, south central Ontario.

slide. Clams were aged under a light microscope by counting the continuous thick, dark bands in a thin section inside the shell. These dark bands represent cessation of a year's growth (Isely 1914; Negus 1966; Haukioja and Hakala 1978; Strayer *et al.* 1981; McCuaig and Green 1983) and were easily distinguishable from noncontinuous thin bands, which are probably formed in response to environmental disturbances. Ghent *et al.* (1978) used a mark-recapture technique to verify growth ring annularity in *Anodonta grandis* and Imlay (1982) concluded from his literature review that aging unionids by counting dark bands within the shells was a very reliable method. In the present study *E. complanata* were independently aged by two people. Results from both were in agreement ($P < 0.05$, $r^2 = 0.70$). Clam ages ranged from 2 to 14 years.

One-way analysis of variance (ANOVA) was employed (using log-transformed trace metal concentrations) to compare trace metal concentrations in gill and body tissue between the two lakes. To assess the interrelationships between clam metal concentrations, canonical variates analyses were also performed on gill and body trace metal data. Analysis of covariance (ANCOVA) was used to compare the relationships between clam age and length and clam metal concentration in the two lakes. Clam length was used as measure of size since it was highly correlated with clam height and width and since it exhibited the greatest range out of these variables.

Results

Trace metal concentrations in both gill and body tissues differed between Beech Lake and Tock Lake clams (Table 2). Tissues of Tock Lake clams had higher concentrations of Cu and Cd and lower concentrations of Zn and Mn than those of Beech Lake clams ($P < 0.05$ in all cases). A canonical variates analysis of trace metal variability in gills defined an axis from Tock Lake to Beech Lake of increasing Cu and Cd concentrations relative to those of Zn and Mn. A similar analysis of trace metal

TABLE 1. Mean concentration (SE) of metals ($\text{mg} \cdot \text{L}^{-1}$) in Tock Lake and Beech Lake

	Tock Lake		Beech Lake
Mn	0.011 (0.001)	*	0.014 (0.001)
Cd	0.0003 ^a		0.0003 ^a
Cu	0.001 ^a		0.001 ^a
Zn	0.008 (0.005)		0.005 (0.002)

NOTE: Samples were collected just above the clam populations in the water column. Water samples (three at each site) were taken at the time of clam collection. Metal analysis was performed by the Ontario Ministry of the Environment.

*Significant difference between lakes ($P = 0.05$).

^aAt or near minimum detectable limits.

TABLE 2. Mean metal concentrations (SE) ($\mu\text{g} \cdot \text{g}^{-1}$ based on dry weight) in gill and body tissues of *E. complanata* from Beech Lake and Tock Lake

	Beech Lake (n = 50)	Tock Lake (n = 50)
Gills		
Cu	13.28(0.80)	18.51(1.11)
Cd	10.10(0.61)	12.78(0.64)
Zn	371.32(15.38)	273.81(10.30)
Mn*	140.80(5.70)	104.90(4.63)
Body		
Cu	7.29(0.26)	10.29(0.98)
Cd	10.98(0.82)	14.51(0.65)
Zn	155.50(6.21)	134.17(8.93)
Mn*	42.20(2.77)	23.50(1.70)

*Mean Mn concentrations have been divided by 100.

TABLE 3. Standardized canonical coefficients for each variable and the correlation of each variable with the canonical score from the canonical variates analyses of gill metal variation and body metal variation

	Gills		Body	
	Standardized coefficient	Structure coefficient	Standardized coefficient	Structure coefficient
Cu	0.771	0.625	0.318	0.428
Cd	0.587	0.417	1.140	0.457
Zn	-1.009	-0.643	0.069	-0.385
Mn	-0.075	-0.590	-1.490	-0.657

variability in body tissues derived an axis from Beech Lake to Tock Lake of increasing Mn concentrations relative to those of Cu and Cd (Table 3). Both models accounted for significant ($P < 0.0001$) proportions of the total variability of trace metals in gill ($r^2 = 0.51$) and body ($r^2 = 0.62$) tissues.

Tock Lake clams were slightly larger (means, 65.2 vs. 61.8 mm; $P < 0.05$) and older (means, 6.4 vs. 4.6 years; $P < 0.05$) than Beech Lake clams. However, there was no relationship between size and age for these populations, possibly because of the great variability in *E. complanata* growth rates among individuals and the lack of extremely young individuals in the samples from both lakes. Size and age were therefore used as independent covariates in each analysis.

Each ANCOVA was successful in predicting tissue trace metal

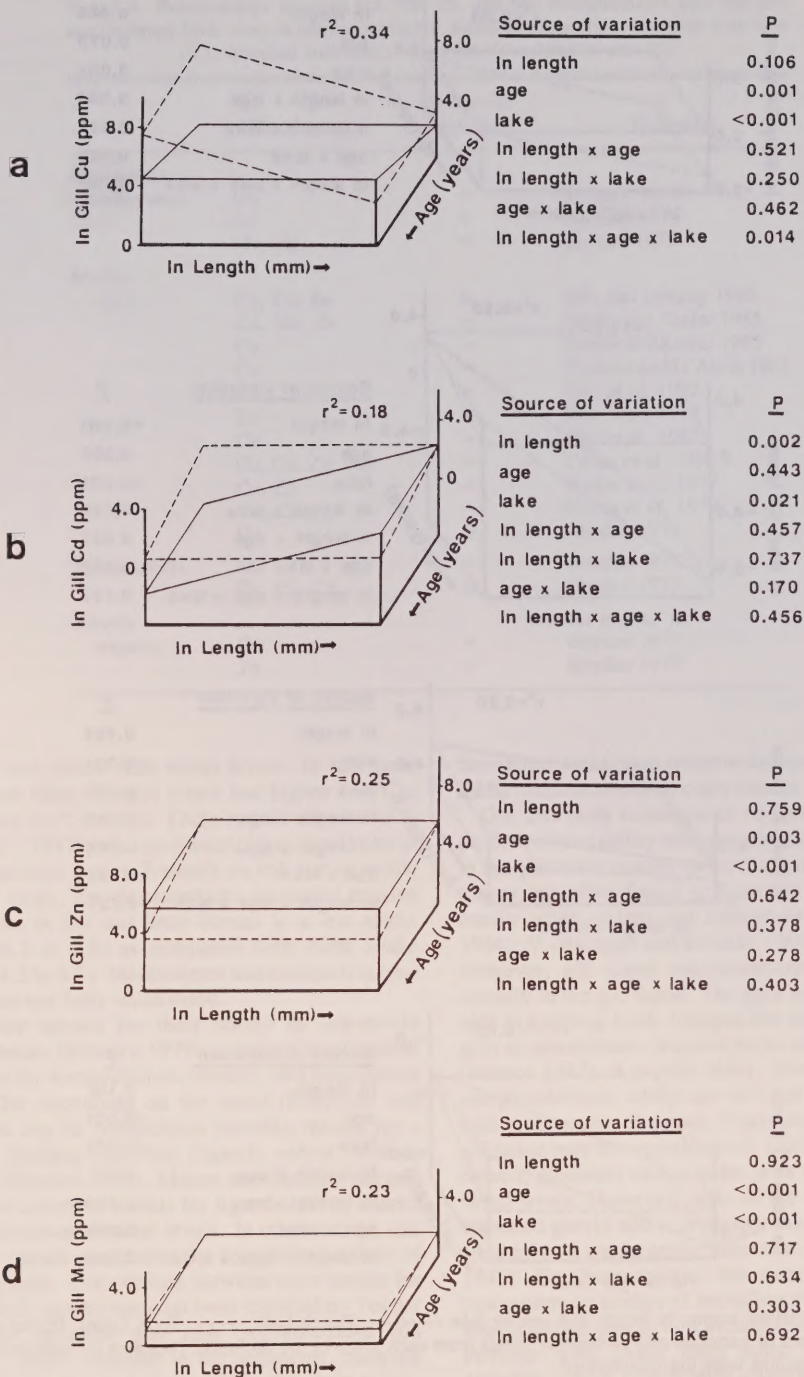


FIG. 2. Gill metal concentrations versus In length and age by lake (—, Beech Lake; ---, Tock Lake). Planes were derived from ANCOVA-predicted values. Source of variation and probability levels from each ANCOVA are included. Negative concentrations may be present because original metal concentrations were log-transformed.

variability and in each case the lake was a significant contributor to the model. Clam age was a good predictor of gill Zn and Mn concentrations (Figs. 2c and 2d) while gill Cd was better predicted by clam size (Fig. 2b). Young, small clams had proportionately higher gill Cu concentrations in Tock Lake than young, small clams in Beech Lake relative to old, large clams

from both lakes (Fig. 2a). Clam age and size were useful in predicting Zn concentrations in body tissues (Fig. 3c) while clam age was primarily responsible for modelling body tissue Mn concentrations (Fig. 3d). Big, old Tock Lake clams had greater body tissue Cu concentrations than small, young Tock Lake clams (Fig. 3a). Young Tock Lake clams had relatively

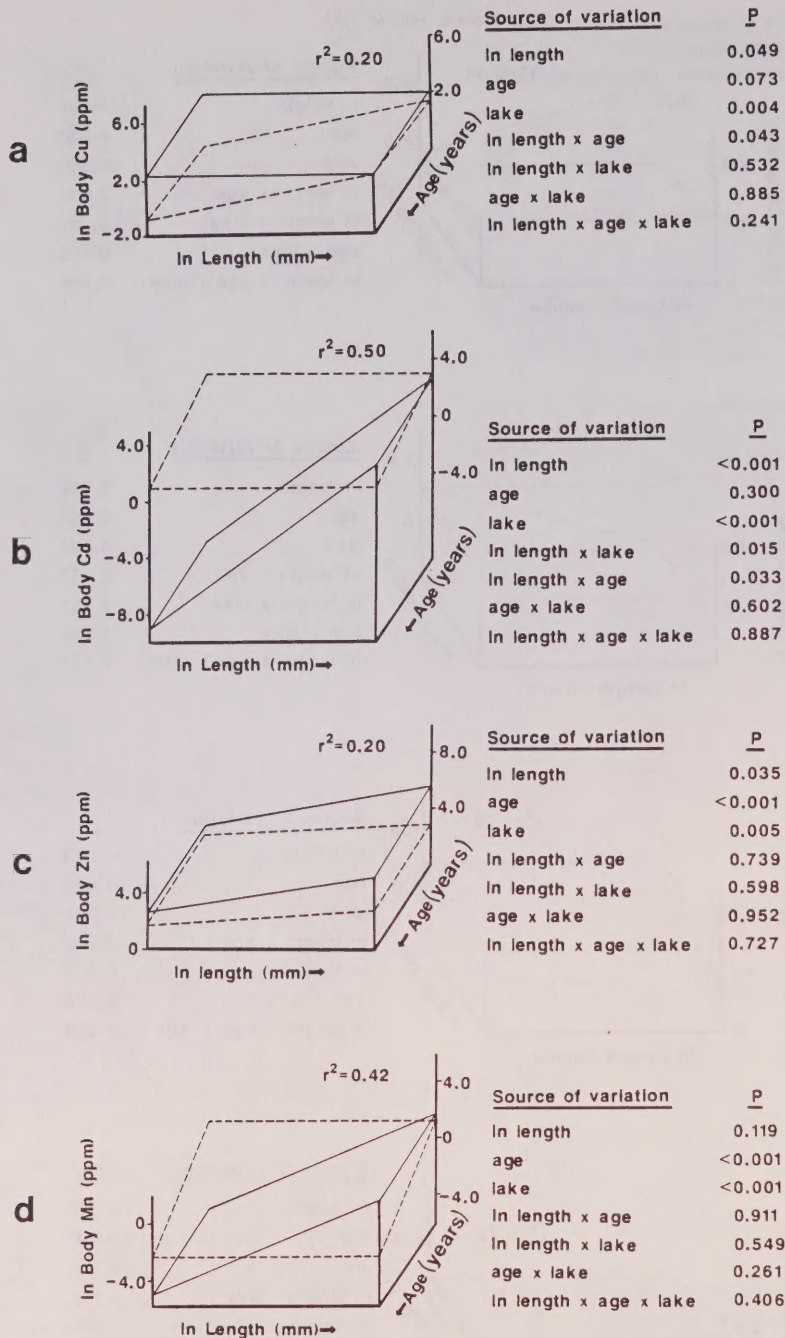


FIG. 3. Body metal concentrations versus ln length and age by lake (—, Beech Lake; ---, Tock Lake). Planes were derived from ANCOVA-predicted values. Source of variation and probability levels from each ANCOVA are included. Negative concentrations may be present because original metal concentrations were log-transformed.

high body tissue Cd concentrations at a given length while large Beech Lake clams showed a similar response at a given age (Fig. 3b).

Discussion

The trace metals examined in this study are known to increase in geochemical mobility (i.e., leave soils and sediments in an aqueous form) with increasing acidification (Tri-Academy

Committee on Acid Deposition 1985) and are generally found in greater concentrations in nonbuffered than in buffered waters in Ontario (Forester 1980). Tock Lake is acid sensitive and has low buffering capabilities while Beech Lake is circumneutral and buffers acidic input. However, water metal levels were similar between the lakes. Although Beech Lake had slightly higher Mn levels in the water than Tock Lake, and Beech Lake clams had higher Mn concentrations than Tock Lake clams, clam metal

TABLE 4. Relationships between Cu, Cd, Zn, and Mn concentrations and size and age (inferred from size) in marine molluscs: metal concentrations increase with size (+), decrease with size (-), or are not related to size (o)

	Trace metal	Metal-size relationship	Reference
<i>Mercenaria mercenaria</i>	Cu	+	Romeril 1979
	Zn	+	Romeril 1979
	Mn, Zn	-	Boyden 1977
<i>Mytilus edulis</i>	Cu, Cd, Zn	o	Brix and Lyngby 1985
	Cd, Mn, Zn	+	Szefer and Szefer 1985
	Cu	-	Szefer and Szefer 1985
	Cu	-	Popham and D'Auria 1983
	Cd	+	Ritz <i>et al.</i> 1982
	Zn	o	Ritz <i>et al.</i> 1982
	Cu	-	Ritz <i>et al.</i> 1982
	Cu, Cd, Zn, Mn	-	Cossa <i>et al.</i> 1980
	Cd, Zn	+	Harris <i>et al.</i> 1979
	Mn, Cu	-	Harris <i>et al.</i> 1979
	Cd	o	Boyden 1977
<i>Ostrea edulis</i>	Mn	+	Boyden 1977
	Zn, Cu, Cd	o	Boyden 1977
<i>Patella vulgata</i>	Cd	+	Boyden 1977
	Zn	-	Boyden 1977

concentrations did not reflect lake metal levels. In the more alkaline environment (Beech Lake) clams had higher Mn/Cu, Mn/Cd, Zn/Cu, and Zn/Cd ratios. These results are similar to those of Servos *et al.* (1987), who examined bioaccumulation of metals by *E. complanata* over a 2-month period during acidic snowmelt. In their study, Zn concentrations increased relative to Cd concentrations in gill and body tissues in a less acidic environment (pH 6.2 to 6.8) as compared with more acidic environments (pH 4.5 to 5.5). Mechanisms that control this type of phenomenon are not fully understood.

Marine clams are known for their ability to selectively accumulate trace metals (Romeril 1979), although mechanisms that are responsible for accumulation, storage, and elimination of metals may differ depending on the metal (Robinson and Ryan 1986). There can be competition between metals for a limited supply of binding substrate (ligand) within mollusc tissue (Mason and Simkiss 1983). Mason and Simkiss suggest that the competitive nature of metals for ligands can be altered depending on environmental metal levels. In other words, one or a few particular metals could alter the competitive nature of many metals for ligands. Competition between trace metals for binding substrate in *E. complanata* has been reported by Tessier *et al.* (1984) who proposed that iron oxyhydroxides and manganese oxides, which originate in the sediments, compete with Cu and Zn for binding substrate within the clam tissue. They suggested that Fe and Mn play a protective role as they may be the principal factors controlling the dissolved trace metal concentrations to which the clam is exposed. Mn may play a similar role in this study. Where Mn levels were relatively low (Tock Lake), Cu and Cd concentrations were greater in the clams. Where Mn levels were relatively high (Beech Lake), Cu and Cd concentrations were lower in the clams. Patterns of clam Zn concentrations cannot be explained in this manner. Metal concentrations in sediment fractions from the lakes may have

been more useful than water metal concentrations for predicting metal concentrations in clam tissues.

Gill and body tissue metal concentrations exhibited similar patterns of variability within each lake, although concentrations in the gills were usually twice those in body tissue. Unionid gill tissues are often found to have the greatest concentration of metals when organs are separately analysed (Tessier *et al.* 1984; V.-Balough and Salanki 1984; Hemelraad *et al.* 1986). However, gill metal concentrations may not reflect what is actually in the gill tissue. The gills are covered in mucus which aids in trapping food. Indigestible material accumulates on the gills as pseudofeces (mucous balls) and is eventually eliminated (Morton 1983). It is quite likely, therefore, that both the mucus and pseudofeces, which are not easily washed off, represent an additional source of metals. Thus concentrations of metals in the gill tissue may be overestimated. During the clams' reproductive season, glochidia raised in the gills may also contribute to gill metal levels. However, none of the clams in the present study appeared gravid and it is known that *E. complanata* generally spawn 4 to 6 weeks earlier than our sampling period (Matteson 1948). For these reasons the current practice in large-scale biomonitoring studies of including gills in whole body analyses should be reassessed. Gill inclusion may be inappropriate if gills increase body tissue trace metal variability. Body tissues probably reflect longer-term environmental metal levels than gills; therefore exclusion of gills from whole body analyses would also allow more accurate comparisons in long-term transplantation studies.

Relationships between metal concentration and clam size and age varied among metals. This type of variability is common in marine molluscs where size-specific metal concentrations vary among and within metals, among and within species, and among studies (Table 4). There are many factors that could enhance variability in size- and age-specific patterns of trace

metals in clams. For example, feeding habits may influence the amount of particulate metals that are ingested (Smock 1983); reproductive state and seasonal variation in growth rate (Strong and Luoma 1981) could alter metal flux by altering metabolic processes (Cain and Luoma 1986) which are responsible for filtering rates (Cossa *et al.* 1980). It is also possible that clam growth could dilute tissue concentrations despite environmental levels if tissue is added faster than metals are accumulated (Strong and Luoma 1981). Physiological explanations for the observed size- and age-specific patterns are not within the scope of this paper. However, it is clear that investigators who wish to compare *E. complanata* metal concentrations should standardize clam size and age. This would especially apply to clam biomonitoring studies where comparisons are made among sites and years.

In only two of eight cases (gill Cu and body Cd) did lake interact with clam size and (or) age in modelling tissue trace metal variability. Buffering capability, which is the primary difference between the two lakes, is therefore not very important in influencing size- and age-specific variability in trace metal concentrations. Popham and D'Auria (1983) came to similar conclusions about *Mytilus edulis*, which were sampled along a pollution gradient. They determined that size- and age-specific metal uptake was independent of population location. However, with only two lakes in our study, caution should be used when our results are extrapolated to larger-scale systems.

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**Isometric patterns of shell etching in clams
from softwater Ontario lakes**

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Abstract

Elliptio complanata (Unionidae: Bivalvia) were sampled from a low and high exposure (water turbulence) site in four softwater Ontario lakes that varied in pH and alkalinity. External shell etching on each clam was quantified using an image analysis digitizer system. Within-lake variation in shell etching was contrasted using a log-log analysis of covariance approach. Clams from seven of the eight sites exhibited isometric shell etching, that is, percent shell etching remained the same as clam size increased. High exposure clams in each lake were proportionately more etched than low exposure clams suggesting that etching is a physical process related to water turbulence. Etching was not related to variation in lake water chemistry.

Introduction

Freshwater unionid clams possess shells which are primarily composed of calcium carbonate (Wilbur 1964). Where calcium and/or carbonate availability is low (i.e. poorly buffered freshwaters) unionid shell growth rates may be reduced (Coker et al. 1921; Green 1980; Singer 1981; Mackie and Flippance 1983). Some unionid species have decreased abundance in low alkalinity environments (e.g., Green 1972), but unionids can be important in a freshwater system's calcium budget. Green (1980) found that Anodonta grandis acted as an important calcium sink in a lake on the edge of the Shield near Inuvik, North West Territories. Approximately 50% of the calcium in this lake was in shells of live A. grandis. This calcium is returned to dissolved form in the lake water only on shell dissolution.

The unionid shell is covered by a thin fibrous schleroproteinaceous layer called the periostracum. This layer is thought to protect the shell from physical and chemical abrasion and to serve as a matrix for the deposition of calcium carbonate crystals (Saleuddin and Petit 1983). The periostracum's resilience seems to decrease on older portions of the shell as it is often missing from the umbonal region. Underlying shell layers, primarily calcium carbonate, are also often missing.

It has been suggested that external shell etching (the loss of periostracum and underlying shell layers) is a chemical process analagous to the weathering of calcareous rock by weak acids (i.e. naturally occuring carbonic acids) (Beauchamp 1886; Grier 1920; Coker et al. 1921). In poorly buffered waters

receiving large inputs of anthropogenic acids, shell dissolution could be enhanced (Coker et al. 1921; Brown and Jewell 1926; Tevesz and Carter 1980). An alternative explanation for this phenomenon is that shells are being physically eroded by contact with abrasive particles in benthic areas experiencing water turbulence. An interaction may exist between physical and chemical etching processes.

The phenomenon of shell etching has never been quantitatively evaluated. In this study we examined shell etching in freshwater unionid mussels (Elliptio complanata) collected from four lakes which vary in alkalinity. Within each lake clams were collected from an area of high exposure (high water turbulence) and low exposure (low water turbulence). Within-lake variation in shell etching due to exposure differences will be contrasted using a log-log analysis of covariance approach. This analysis also enables one to examine how shell etching varies with clam size. The results from these analyses for the four lakes will be compared.

Methods

The four study lakes are located in south-central Ontario on the Precambrian Shield (Fig. 1). Although morphologically similar (see Hinch and Bailey, In press), the lakes were chosen to differ in alkalinity and pH. Mean wind direction for the ice free season is from the west-south-west, based on Environment Canada wind summary data from 1983 and 1984. In August 1985, twenty-five clams were collected using SCUBA from a low (southwest) and a high (northeast) exposure site in each lake (Fig. 2). Clams ranged from 1.5 to 2.5 m depth. Stretches of shoreline near inflows and outflows were not sampled. To reduce the effects of age structure as a confounding factor only old individuals (greater than 8 years of age based on annual rings) were collected. These clams are very near their asymptotic sizes.

The meat was removed from each clam and the shells allowed to dry. Shell etching was quantified using an image analysis process run on a microcomputer-based digitizer system. Detailed descriptions of the design, construction, specifications and software of the system can be obtained through correspondence with Dr. Lewis Brown (Dept. Plant Sciences, University of Western Ontario, London, Ontario, Canada; unpubl. data). Shell etching was defined as any area on the shell which was missing periostracum and underlying shell layers (Fig. 3). In preparation for the digitizing process, all etched areas were painted white. This provided a sharp contrast with the dark brown non-etched areas. Non-etched shell areas were first determined. Both valves of a given shell were placed under the video camera, with the outer shell surface up. The dark area was digitized and

its area determined. Although this digitizing process only determines area in 2 dimensions, E. complanata have a thin profile (Hinch and Bailey in press). Therefore distortion caused by shell width was probably minimal. Total shell area was then determined by painting the shell's entire external surface black and repeating the digitizing process. Etched area was equal to the total shell area minus the non-etched shell area.

Investigating the differences in etched shell area between high and low exposure clams among the four lakes is the purpose of this study. However, comparing absolute differences would ignore variability attributable to clam size. Since etched area may vary with size, some form of standardization is required. Deriving a ratio variable (i.e. percent shell area etched = $\text{etched area} / \text{total area} \times 100$) would not be useful since ratios possess poor statistical properties and they tend to obscure certain relationships when used as dependent variables in statistical models (see Green 1979, pp. 104-105; and references within). A log-log regression analysis using log of etched area (LEA) as the dependent variable and log of non-etched area (LNEA) as the independent variable is more suitable in this situation (Green 1986). With this statistical model the following scenarios would apply. A regression slope of one would occur when percent etched area remains unchanged as clam size increases. A slope greater than one implies that larger clams have a greater percentage of the shell etched than do smaller clams, and a slope less than one implies that larger clams have a lower percentage of the shell etched.

It could be argued that the independent variable should be some measure of clam size (i.e. total etched area or clam length) and not LNEA. However, interpreting these results would be difficult because LEA is included in clam size. This would be analogous to regressing variable 'Y' on variable 'X + Y' which could result in spurious relationships caused by variable confounding.

Analyses of covariance (ANCOVA), with log of non-eroded area as the covariate, were used to compare regression relationships between the high and low exposure sites within each of the four lakes. A nested, random effects ANCOVA, similar to the preceding model except that among-lake variation is also estimated, was used to estimate the proportion of the total variance in shell etching attributable to variation among the four lakes, between exposure level sites within the lakes, and among individual clams within the sites.

Results

Bonferroni's inequality, which controls the maximum experimentwise error rate (Snedecor and Cochran 1980), was applied to the ANCOVA results for the four lakes. The criterion error rate for each of four simultaneous comparisons is $P < 0.013$, in order to obtain an experimentwise error rate of 0.05. Comparisons of the interaction term (LNEA X SIDE) from the four analyses revealed that all were non-significant except for Bark Lake which was marginally significant (Table 1). Thus, only in Bark Lake is there evidence that exposure influences the relationship between clam size and percentage of shell that is etched. The effect of exposure (SIDE) was significant in each lake indicating that exposure influences the percentage of shell that is etched. The high exposure clams were proportionately more etched than the low exposure clams in each lake (Table 2).

The hypothesis that the slope of LEA-LNEA relationships is one cannot be rejected for clams from any of the eight sites except for those from the high exposure Bark Lake site, which exhibit a slightly negative LEA-LNEA relationship (Table 3). This is based on Bonferroni's inequality, the criterion error rate for eight simultaneous comparisons being $P < 0.0064$, in order to obtain an experimentwise error rate of 0.05.

Components of variance computed from the nested ANCOVA indicate that 52.3% of the total shell etching variability resulted from differences between exposures (within-lake). Lakes did not differ significantly in degree of shell etching. The remaining 47.7% was accounted for by differences among individual clams within sites.

Discussion

Shell etching does not appear to be related to differences in lake water chemistry (i.e. alkalinity, pH) as others have proposed (Grier 1920; Coker et al. 1921; Tevesz and Carter 1980). Since variability in etching was primarily explained by exposure differences, we suggest that shell etching is mostly a physical process related to water turbulence. Shells probably are eroded either by abrasive sediment particles colliding with the shell or by shells being dislodged and scoured on the substrate. Shell etching may be greater on clams from exposed habitats because larger and coarser sediment particles are more prevalent in wave-washed, turbulent habitats. This "sand-blasting" effect could weaken the proteinaceous periostracum of the shell until it begins to flake off. Underlying the periostracum are prismatic and nacreous shell layers. These layers consist primarily of organic material (conchiolin) and calcium carbonate (Tevesz and Carter 1980). Once exposed, the calcium carbonate would be vulnerable to dissolution. Total shell dissolution is prevented or at least slowed down by thick, adventitious organic layers which are sporadically distributed within the nacreous layers. Although these layers can be actively deposited in response to deep solution pitting in certain unionids, their effectiveness will presumably be reduced by continued shell erosion.

The slopes of the LEA-LNEA regressions did not differ from one (except for Bark Lake high exposure clams) implying isometric changes between shell etching and clam size. Percent shell etching remains the same as clams size increases for both high and low exposure clams despite the fact that high exposure clams

from each lake have a much faster growth rate than the corresponding low exposure clams (Hinch and Bailey in press).

The nature of the size-etching relationship in high exposure Bark Lake clams is different from the other sites. In these clams, non-etched area increases at a faster rate than etched area decreases. Thus it would appear that as clams get bigger, absolute shell etching gets smaller. Although unionids can superficially reseal holes in their shells and can reinforce eroded areas using adventitious conchiolin (Tevesz and Carter 1980) it is unlikely that they can replace nacreous shell layers and periostracum on their umbo. We cannot explain these types of etching patterns.

Log-log ANCOVA models are useful for estimating and comparing percentages (i.e. percent shell erosion from low and high exposure clams in the present study) without using derived ratio variables (Atchley et al. 1976; Green 1979, pp. 103-105; Green 1986). There may be a problem with application of ordinary least squares (OLS) regression to such "error-in-X" bivariate data. Estimates of slopes may be biased downward (Green 1986). For example, the low slope values estimated for some of the eight sites may not really be that low. McArdle (in press) discusses complementary and alternative methods to performing OLS regression on these data.

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Table 1. Probability levels for main and interaction effects from the analyses of covariance contrasting low and high exposure sites within the four lakes. For an experimentwise error rate of $\alpha=0.05$, the appropriate α for each test is 0.013 based on Bonferroni's inequality. Astericks denote values that are not associated with null hypotheses of interest.

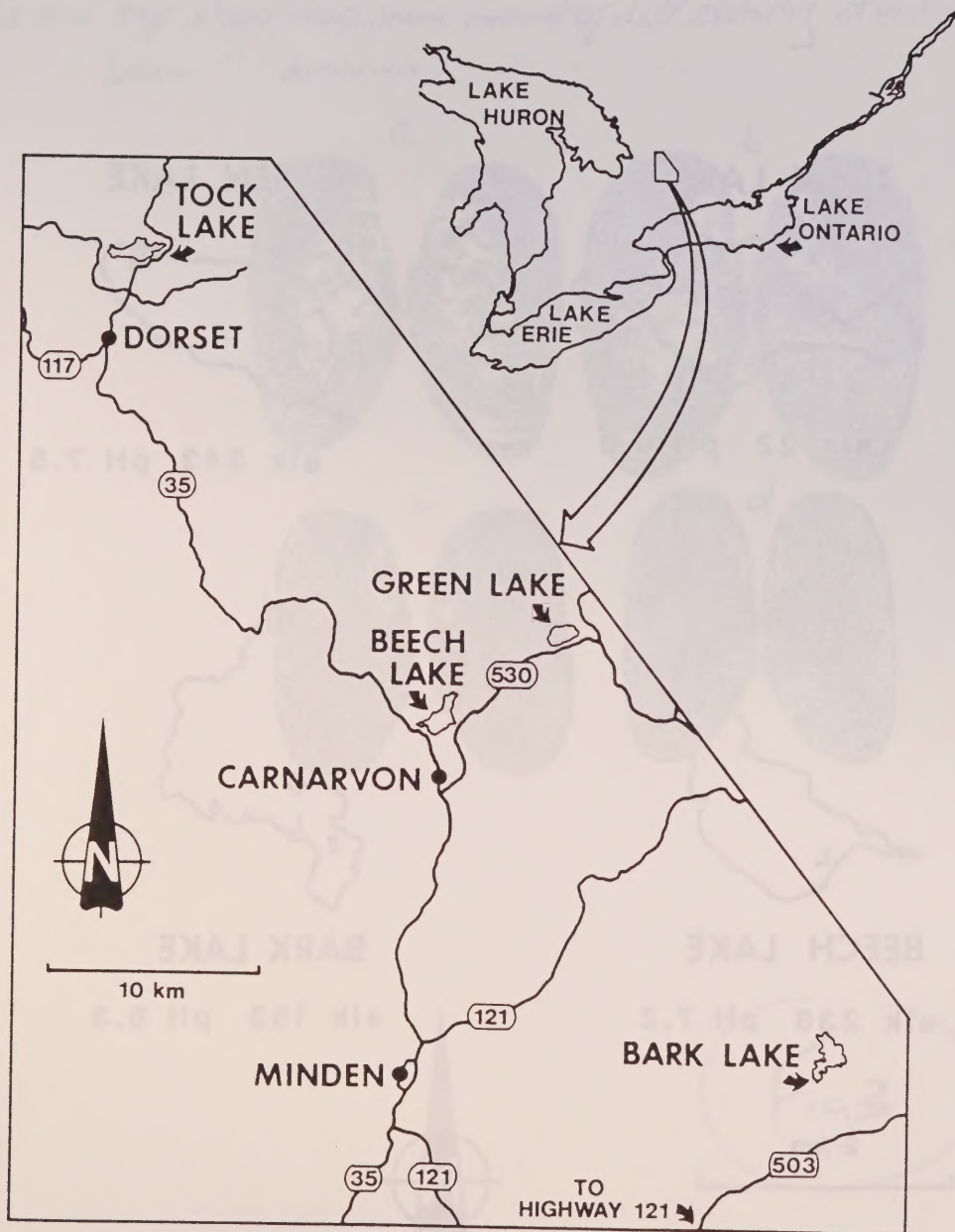
	Beech Lake	Green Lake	Bark Lake	Tock Lake
log-non-etched area (LNEA)	*	*	*	*
side of lake (SIDE)	<0.001	<0.001	0.009	<0.001
LNEA X SIDE	0.024	0.267	0.008	0.022

Table 2. Mean (SE) shell etched area (mm^2) from the two sites within the four lakes. At each site $n=25$.

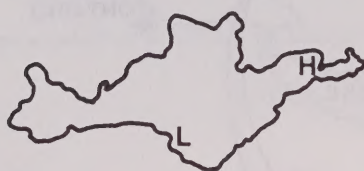
site	mean	SE
Beech high	1216.83	(98.43)
Beech low	429.08	(76.65)
Green high	638.03	(77.47)
Green low	277.74	(34.95)
Bark high	475.84	(45.55)
Bark low	368.86	(26.05)
Tock high	801.77	(57.95)
Tock low	300.33	(29.33)

Table 3. Estimates of slope (SE) for LEA-LNEA regression relationships from the two sites within the four lakes. The t-statistics are based on the null hypothesis that slope = 1. Absolute t values are listed since test is two-tailed. The critical t value (df=23; $\alpha=0.05$) is 2.07. For an experimentwise error rate of $\alpha=0.05$, the appropriate α for each test is 0.0064 based on Bonferroni's inequality. The critical t value (df=23; $\alpha=0.0064$) is 3.26.

site	slope	SE slope	t value
Beech high	-0.46	0.74	1.97
Beech low	2.49	1.04	1.43
Green high	1.72	0.80	0.90
Green low	0.86	0.27	0.52
Bark high	-1.81	0.57	4.90
Bark low	0.34	0.54	1.20
Tock high	1.30	0.41	0.73
Tock low	-0.78	0.79	2.25

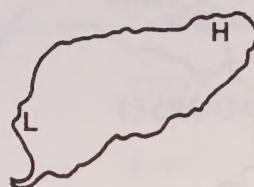


TOCK LAKE



alk 22 pH 6.0

GREEN LAKE



alk 243 pH 7.5



BEECH LAKE

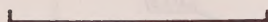
alk 238 pH 7.2



BARK LAKE

alk 153 pH 6.3

2 km



This is a photograph of clamshells from Beech Lake.
showing variability in shell etching.

Shells on the left are from the low exposure site
right high exposure site

Shells on top show maximum amount of shell etching at each site.
 " " bottom " minimum " " " " " " " " " " " "

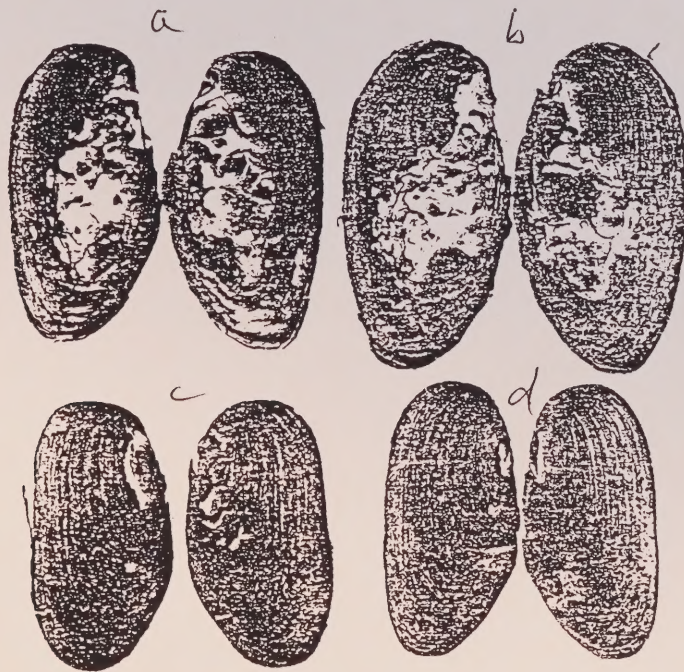


Fig 3

Control No. ECD125

AGING AND CONTAMINANT ANALYSIS OF
MUSSEL SHELLS FROM THE SHUBENACADIE RIVER
HEADWATER LAKES IN NOVA SCOTIA

by

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EXECUTIVE SUMMARY

Freshwater mussels (clams) from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg). Soft tissue concentrations of these elements vary among species and lakes, and are influenced by body weight. Age is also suspected to be a factor, therefore the shells of three species of mussels from two lakes (n=249) were aged. One of the species (Elliptio complanata) could be aged much more accurately than the other two (Anodonta implicata and Alasmidonta undulata). It also grew more slowly and was longer-lived (17 years or more vs. 8-9 years for the other species). E. complanata from the most contaminated site (Powder Mill Beach) were significantly smaller at a given age than those from the less contaminated Lake Thomas, suggesting the possibility of sublethal toxic effects. The influence of age on differences in the bioaccumulation of As and Hg among the species could not be determined, because age estimates for A. implicata and A. undulata were too inaccurate.

E. complanata is recommended for biomonitoring in the Atlantic region. Because it is long-lived, contaminants should bioaccumulate over time to detectable levels. Also, because it can be accurately aged, different year classes can be analyzed separately. This species is widely distributed in central and eastern Canada, and has been used extensively for biomonitoring in Ontario and Quebec. Therefore, there is an existing data base for comparing environmental contamination in the Atlantic region with other regions in Canada.

Mussels accumulate metals in their shells as well as their soft tissues. Shells retain a sequential record of contamination in the annual growth rings, and therefore have potential for mapping changes in environmental contamination over time. Two techniques for analyzing trace metals in shells, the electron microprobe and secondary ion mass spectrometry (SIMS), were evaluated. Both failed to detect measureable concentrations of As, Hg, Pb or Cd due to a lack of sensitivity and/or various interferences. These methods require further development and refinement before they can be used for this application.

This work was supported by DSS contracts to the University of Western Ontario, and was partly funded by the Water Quality Branch, Atlantic Region.

MANAGEMENT PERSPECTIVE

The freshwater mussel, Elliptio complanta is recommended for biomonitoring in the Atlantic region, based on its high bioaccumulation capacity for environmental contaminants, its longevity, the accuracy with which it can be aged, and its apparent sensitivity to toxicants. In earlier work, we found that E. complanata from the Shubenacadie River headwater lakes bioaccumulated high concentrations of arsenic and mercury in their soft tissues. This species has also been shown to bioconcentrate other heavy metals and organochlorine pesticides. In the present study we found this species to live almost two decades - twice as long as two other species with which it coexists in the Shubenacadie watershed. E. complanata is therefore capable of accumulating persistent contaminants over long periods of time to analytically detectable levels. Because it can be accurately aged, which was not the case for the other two species, relationships between age and contaminant uptake can be confidently evaluated. Furthermore, age classes may be analyzed separately in order to identify trends in environmental contamination. E. complanata from Powder Mill Beach, which is highly contaminated with arsenic and mercury, were significantly smaller at a given age than those from the less contaminated Lake Thomas. This suggests the possibility of sublethal toxic effects.

Mussels accumulate contaminants in their shells as well as their soft tissues. Shells retain a sequential record of contamination in

their annual growth rings, and therefore have potential for mapping changes over time. Two techniques for analyzing trace metals in growth rings, the electron microprobe and secondary ion mass spectrometry (SIMS), were evaluated. Both failed due to a lack of sensitivity and/or various interferences. With further development and refinement, however, the SIMS technique shows promise.

ABSTRACT

Freshwater mussels from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg) due to historic gold mining activities in the area. Soft tissue concentrations of these elements vary among lakes, species and size classes, and are suspected to be influenced by age. To evaluate this influence, which is the topic of a later report and to compare longevities and growth rates among species and lakes, the shells of three species of mussels from two lakes were aged. Specimens of Elliptio complanata could be aged much more accurately than either Anodonta implicata or Alasmidonta undulata. They also grew more slowly, lived longer, and were significantly smaller at a given age at the most contaminated site, which suggests the possibility of a sublethal toxic response. E. complanata is recommended for biomonitoring in the Atlantic region based on its above-described characteristics, as well as its previously demonstrated high bioconcentration capacity for environmental contaminants and its widespread distribution.

Mussels accumulate contaminants in their shells as well as their soft tissues. Shells retain a sequential record of contamination in their annual growth rings, and therefore have potential for mapping changes over time. Two techniques for analyzing trace metals in growth rings, the electron microprobe and secondary ion mass spectrometry, were evaluated. Both failed due to a lack of sensitivity and/or various interferences.

GENERAL INTRODUCTION

Bivalve molluscs have been shown to be excellent organisms for biomonitoring contaminants in aquatic systems. They accumulate and concentrate a wide range of environmentally available pollutants and they are widely distributed both globally and in terms of the habitats they occupy (Green et al, 1985). Marine bivalves have been extensively used to study the occurrence and bioavailability of metals, hydrocarbons and radionuclides in marine environments (Goldberg et al, 1978; Burns and Smith, 1981; Koide et al, 1982). In recent years, the freshwater unionid mussels have received some attention as biomonitors for metals (Smith et al, 1975; Manly and George, 1977; Tessier et al, 1984), chlorinated organic contaminants (Kauss and Hamdy, 1985) and pesticides (Miller et al, 1966; Bedford et al, 1968).

Unionaceans meet many of the criteria for a biomonitoring organism, as summarized by Phillips (1977) and Forester (1980). They accumulate a wide variety of substances without being killed, and are large enough to provide adequate tissue for residue analysis. Because of their low position in the food chain, they provide a direct measure of bioavailability. Finally, they are long-lived. In fact, freshwater mussels probably live the longest among freshwater invertebrates (Imlay, 1982). Their longevity allows for sampling more than one year class, and integrates environmental conditions over long periods of time. A further characteristic of mussels, their amenability to relocation, was considered important by Klumpp and Burdon-Jones (1982).

Resident freshwater mussels from the Shubenacadie River headwater lakes in Nova Scotia were found to have excellent potential as biomonitors for arsenic and mercury (Metcalf and Mudroch, 1985). Contamination of the lake chain is due to historic gold mining activities in the area. Recent urban and industrial expansion has disturbed the mine tailings and bedrock, and is believed to have aggravated an already existing contamination problem. Mussels may provide a record of past disturbances in the system as well as a means of monitoring the ecological consequences of further development or ameliorative measures.

PART A: AGING OF MUSSEL SHELLS

INTRODUCTION

Metcalf and Mudroch (1985) found that mussels from the Shubenacadie Lakes were extraordinarily contaminated with arsenic (up to 117 $\mu\text{g/g}$ dry weight) and mercury (up to 9.95 $\mu\text{g/g}$). Site to site differences were suggested and have since been verified (Metcalf and Mudroch, unpublished data). Interspecific differences in the bioaccumulation of mercury were apparent, despite the fact that all species are deposit feeders. There was a relationship between the size (dry weight) of the organism and the bioaccumulation of both

elements, but particularly mercury. Because weight and age are highly correlated ($r = 0.67 - 0.87$ for the three resident species), we suspect that age is an important factor influencing the bioaccumulation capacity of mussels.

Mussels can be aged by counting the annual "winter" rings on the surface of their shells which mark the end of each growing season. Because of the occurrence of "pseudoannual" disturbance bands, which could be mistaken for annual rings, age may be verified by preparing thin sections of the shells and counting the internal bands. This technique was used to age mussels which had been collected from two of the most contaminated lakes in the Shubenacadie watershed for analysis of arsenic and mercury residues in their soft tissues.

This report presents the ages of the specimens, and evaluates the accuracy of the aging procedure for each species. The results are used to compare growth rates and longevity among species and between lakes. This information will be used to determine the relationship between age and the accumulation of arsenic and mercury by mussels from the Shubenacadie Lakes.

MATERIALS AND METHODS

Description of Study Area

The Shubenacadie Basin is located north of Halifax, Nova Scotia (Fig. 1). Gold mining, which centred around the village of Waverley at the turn of the century (Trip and Skilton, 1985), is the source of

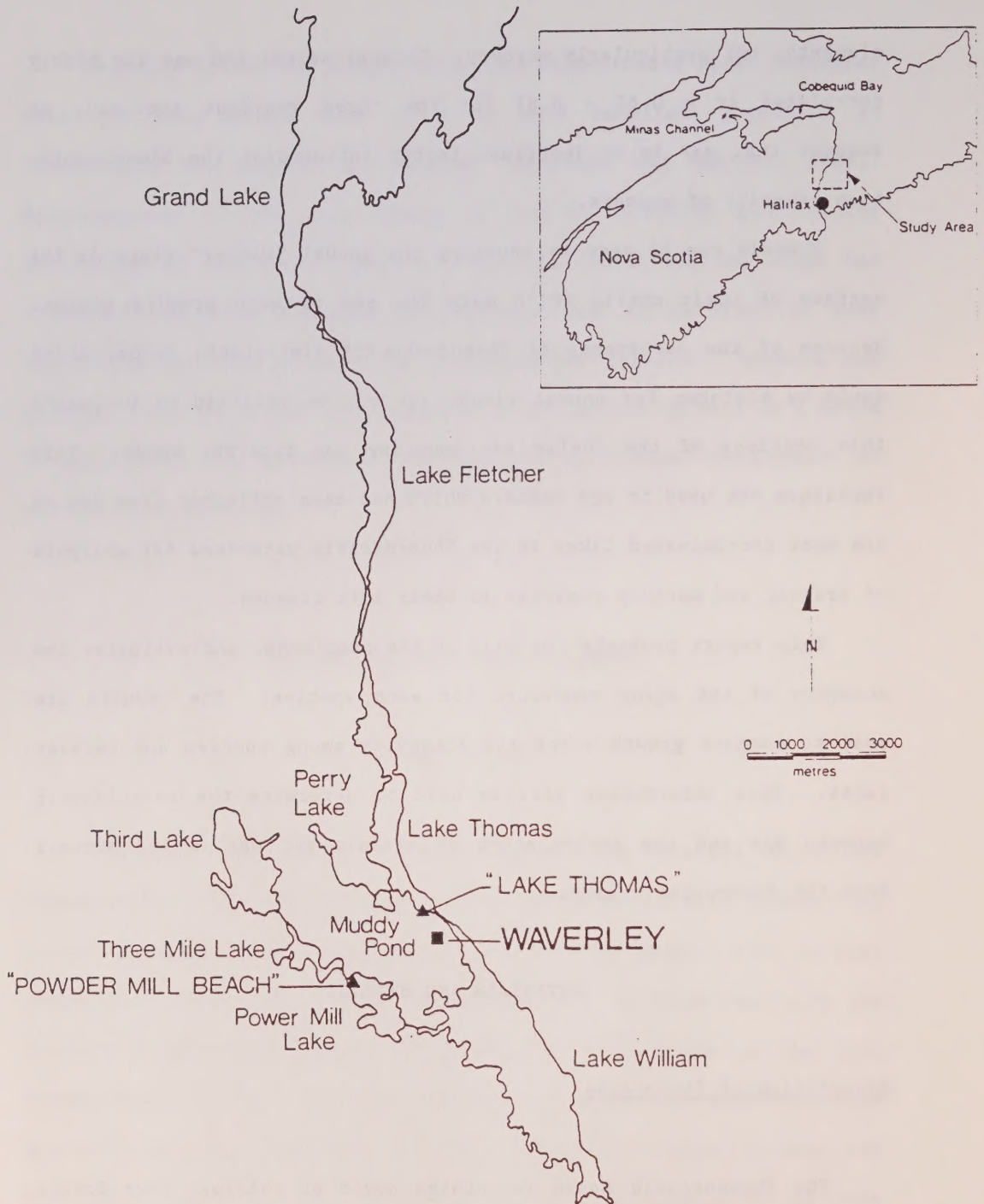


Figure 1. LOCATIONS OF MUSSEL COLLECTION SITES
"LAKE THOMAS" AND "POWDER MILL BEACH".

arsenic and mercury contamination in the watershed. The lakes closest to Waverley, especially Powder Mill Lake, Lake Thomas, Muddy Pond and Lake William, are the most contaminated. Large beds of unionid mussels occur in the nearshore areas of the former two lakes at the sites were referred to as "Powder Mill Beach" and "Lake Thomas" (Fig. 1). Relative contamination of the water, suspended sediment and bottom sediment at these two sites are compared in Table I. The data indicate that Powder Mill Beach is more contaminated with both arsenic and mercury. The substrate at Powder Mill Beach was sandy and was easily sampled, whereas at Lake Thomas the substrate was rocky and a representative sediment sample could not be obtained. However, we would expect the sediment at the Lake Thomas site to be less contaminated because this is certainly true for the deposition zone.

Collection of Mussels and Preparation of Shells

Mussels were collected from shallow areas (<1 m depth) at the two sites on July 31, 1984 and July 30, 1985. A total of 249 specimens of the following three species were obtained: Alasmidonta undulata (Subf. Anodontinae), Anodonta implicata (Subf. Anodontinae) and Elliptio complanta (Subf. Ambleminae). Table II presents the numbers of each species collected from each site.

In 1984, mussels were frozen whole on the day they were collected. A week later in the laboratory they were thawed and shucked, and the soft parts were prepared for quantitative determination of arsenic and mercury. In 1985, mussels were shucked

TABLE I. Relative contamination of the Powder Mill Beach and Lake Thomas sites with arsenic and mercury (from Metcalfe and Mudroch, 1985).

Compartment	Powder Mill Beach		Lake Thomas	
	As	Hg	As	Hg
Water ($\mu\text{g/L}$) ¹	0.2-4.1	ND(<0.02)	2.1-2.6	ND(<0.02)
Suspended Sediment ($\mu\text{g/g}$) ¹	288-319	2.25-3.30	129-490	.46-.74
Nearshore Bottom Sediment ($\mu\text{g/g}$; 0-2 cm)	890-3050 ²	5.69 ³	NS	NS
Deposition zone bottom sediment ($\mu\text{g/g}$; 0-10 cm)	749	20.8	163	10.5

¹Seasonal ranges

²Range of five samples

³One sample

NS = not sampled; substrate rocky

TABLE II. Numbers of mussels of each species collected from each site.

Species/Site	Year		Total
	1984	1985	
A. <u>undulata</u>			
Powder Mill Beach	10	20	30
A. <u>implicata</u>			
Powder Mill Beach	20	73	93
Lake Thomas	18	26	44
E. <u>complanata</u>			
Powder Mill Beach	5	10	15
Lake Thomas	13	54	67
			249

fresh on the day of collection. All shells were air-dried and weighed to the nearest milligram. Valve lengths were measured to the nearest 0.05 cm using vernier calipers. The shells were stored in plastic bags until they could be sectioned for aging.

Aging Procedure

For each species at each site, about 12 (10-13) specimens representing the range of size classes present were selected for sectioning. All specimens were taken from the 1985 collection. Following the methods of Clark (1980) and Kennish et al (1980), the right valves of each specimen were coated with epoxy, cut along the axis of maximum growth, mounted on a microscope slide, then further cut to produce a thin section. The internal bands, each of which corresponds to a year's growth, were then counted independently by two "evaluators". External rings were also counted for *A. implicata* only. The reliability of aging methods based on the annular rings of unionids has been well established, as reviewed by McCuaig and Green (1983).

Statistical Analysis

Accuracy of the aging procedure was evaluated by calculating the degree of correlation, as measured by the correlation coefficient " r ", between the two independent age estimates for each series of specimens. These independent age estimates were then averaged, and

the best regression model predicting valve length as a function of age and site was determined. The "best" model was that in which no significant predictors were omitted but no nonsignificant predictors were included. Site could not be included as a predictor for A. undulata, since all specimens came from Powder Mill Beach. Valve lengths were then used to estimate the ages of the remaining specimens which had not been sectioned. Shell weight was also evaluated as a predictor for age.

RESULTS

Elliptio complanata

The valve lengths, shell weights, and age estimates from internal bands for the 22 specimens which were sectioned are presented in Appendix IA.

Age estimates by the two independent evaluators were found to be highly correlated ($r = .835$), and this relationship was statistically significant ($t = 6.79 > t_{20} = 2.086$; $p \leq .05$). The regression equation was: $y = -0.592 + 1.06 x_1$, where y = age estimate by evaluator #1 and x_1 = age estimate by evaluator #2. The slope of the line was not significantly different from 1.0 ($t = .38 \nless t_{20} = 2.086$), nor was the intercept significantly different from zero ($|t| = .35 \nless t_{20} = 2.086$), therefore both evaluators saw the same number of internal bands in a given specimen and the aging procedure can be considered to be very accurate for this species.

The "modelling run" for predicting valve length was initiated with the following model:

$$\hat{y} = a + bx_1 + bx_2 + bx_3 + bx_4.$$

where: $\hat{y}$ = predicted valve length (cm)
 a = intercept
 b = slope coefficients
 x_1 = age (average of two estimates)
 x_2 = site (Lake Thomas = 1; Powder Mill Beach = 2)
 x_3 = (age)²
 x_4 = (age)(site) interaction

The latter two predictors were not significant and the best fitting regression line for predicting valve length for E. complanta was:

$$\hat{y} = 7.9965 + 0.30082x_1 - 1.8096x_2; \quad r = .88$$

where the effects of both age and site were significant (age: $t = 5.36 > t_{1,} = 2.093$; site: $|t| = 4.80 > t_{1,} = 2.093$. Thus, mussels from Lake Thomas were larger at a given age than those from Powder Mill Beach. This model was rearranged, as shown below, in order to solve for $\hat{x}$ (estimated age):

$$\hat{x} = y - \frac{7.9965 + 1.8096x_2}{0.30082}$$

The equation was then used to estimate the ages of the remaining 60 specimens which had not been sectioned and for which counts of internal bands had not been made (Appendix IB). The effect of site on mussel size is best illustrated by plotting the regression equations predicting valve length from age for each site individually (Fig. 2). These equations are:

Lake Thomas: $\hat{y} = 6.5435938 + 0.2682652x_1; n = 12; r = .73.$

Powder Mill Beach: $\hat{y} = 3.8800644 + 0.3534323x_1; n = 10; r = .85.$

The slopes of these two lines did not differ significantly ($F = .52$ } $F_{1,18} = 4.41$), therefore the growth rates of mussels five years of age and older do not differ between the sites. Because the intercepts differ, it is apparent that mussels at the Lake Thomas site must grow faster during the early age period (less than five years).

Shell weight was also evaluated as a predictor for age. The best model was:

$$\hat{y} = 17.413 + 2.5059x_1 - 11.711x_2; r = .80.$$

where: $\hat{y}$ = shell weight (g)

x_1 = age (average of two estimates)

x_2 = site (Lake Thomas = 1; Powder Mill Beach = 2).

Length was a better predictor of age ($r = .88$) than weight was ($r = .80$).

Anodonta implicata

The valve lengths, shell weights, age estimates from internal bands, and age estimates from external rings for the 25 specimens which were sectioned are presented in Appendix IIA.

This is the only species for which both internal and external rings were counted. Age estimates by the two independent evaluators were found to be highly correlated for the external counts ($r = .830$), and moderately correlated for the internal counts ($r = .586$). The latter relationship was, nevertheless, statistically significant ($t = 3.47 > t_{2,} = 2.069$). Both evaluators saw more external than internal rings. The regressions of external counts (y) on internal counts (x) for each evaluator are as follows:

$$\text{Evaluator \#1: } \hat{y} = 1.4766484 + 0.8770604x; \quad r = .71.$$

$$\text{Evaluator \#2: } \hat{y} = -0.4575045 + 1.2495479x; \quad r = .69.$$

The evaluators differed in that #1 saw more external rings in younger specimens (i.e., for $x = 4$, $\hat{y} = 5.0$ and for $x = 10$, $\hat{y} = 10.2$), while #2 saw more external rings in older specimens (i.e., for $x = 4$, $\hat{y} = 4.5$ and for $x = 10$, $\hat{y} = 12.0$).

The model for predicting valve length was based on average internal band counts, despite the fact that there was more agreement between the evaluators in their external ring counts. The "modelling run" was initiated with the same model as for *E. complanta*. In this case, however, there was no site effect and the best fitting regression line for *A. implicata* was:

$$\hat{y} \text{ (valve length)} = 1.102 + 1.0710x_1 \text{ (age)}; \quad r = .70.$$

where the effect of age was significant ($t = 4.64 > t_{2,} = 2.069$). This model was rearranged, as shown below, in order to solve for $\hat{x}$ (estimated age):

$$\hat{x} = \frac{y - 1.102}{1.0710}$$

The equation was then used to estimate the ages of the remaining 112 specimens which had not been sectioned and for which counts of internal bands had not been made (Appendix IIB). Although site did not influence the length/age relationship, regression equations predicting valve length from age were calculated separately for each site, allowing comparisons with the other two species (Fig. 2). These equations are:

Lake Thomas: $\hat{y} = 2.6571154 + 0.7988462x_1; \quad n = 12; \quad r = .59.$

Powder Mill Beach: $\hat{y} = -0.8312896 + 1.4211416x_1; \quad n = 10; \quad r = .81.$

Shell weight was also evaluated as a predictor for age. The best model was:

$\hat{y} = -19.142 + 4.946x_1$; $r = .66$. Where y = shell weight (g) and x_1 = age

Length was a better predictor of age ($r = .70$) than weight was ($r = .66$).

Alasmidonta undulata

The valve lengths, shell weights, and age estimates from internal bands for the 12 specimens which were sectioned are presented in Appendix IIIA.

Age estimates by the two independent evaluators were not strongly correlated ($r = .536$), and in fact the relationship was not statistically significant ($t = 2.01$ } $t_{1,0} = 2.228$). Despite this disagreement, the model for predicting valve length was based on averaged internal band counts. The model could not include site as a predictor because A. undulata only occurred at Powder Mill Beach, therefore the "modelling run" excluded the x_2 and x_4 components of the original model (site, and age x site interaction, respectively). The effect of x_3 , $[(age)^2]$ was not significant, therefore the best fitting regression line was:

$$\hat{y} = 0.8398 + 0.5182x_1; r = .80.$$

where the effect of age was significant ($t = 4.24 > t_{1,0} = 2.228$). This regression line is plotted in Fig. 2 for comparison with the other two species. The model was rearranged, as shown below, in order to solve for x (estimated age):

$$\hat{x} = \frac{y - 0.8398}{0.5182}$$

The equation was then used to estimate the ages of the remaining 18 specimens which had not been sectioned and for which counts of internal bands had not been made (Appendix IIIB).

Shell weight was also evaluated as a predictor for age. The best model was:

$$\hat{y} \text{ (shell weight)} = -5.409 + 1.5627x_1 \text{ (age)}; r = .83.$$

This was the only species for which shell weight was a slightly better predictor of age ($r = .83$) than valve length was ($r = .80$).

Interspecific Comparisons

The relationships between valve length and age for each species at each site are compared in Fig. 2. As previously mentioned, E. complanta from Lake Thomas were larger at a given age than those from

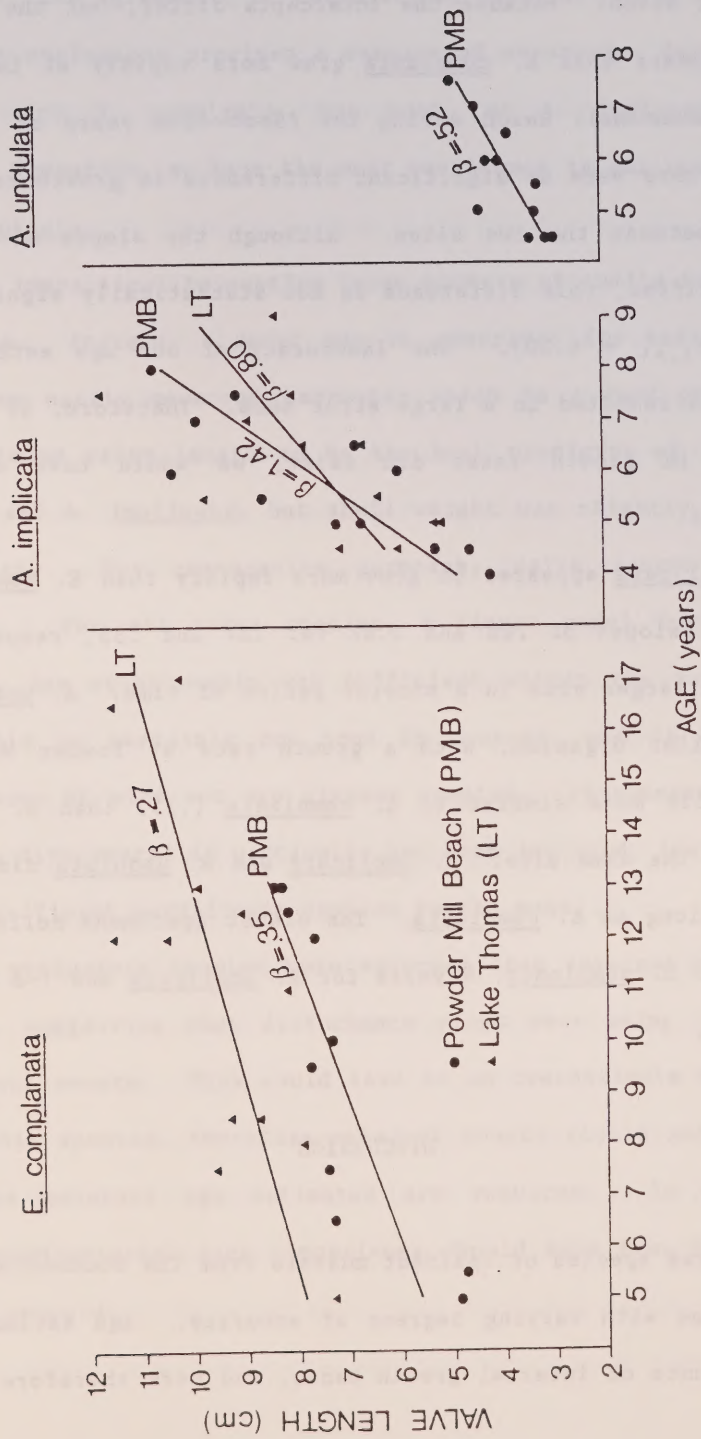


Figure 2. REGRESSION EQUATIONS PREDICTING VALVE LENGTH AS A FUNCTION OF AGE FOR EACH SPECIES OF MUSSEL AT EACH SITE.

Powder Mill Beach. Because the intercepts differ, but the slopes do not, it appears that *E. complanta* grew more rapidly at Lake Thomas than at Powder Mill Beach during the first five years of life. In contrast, there were no significant differences in growth rates of *A. implicata* between the two sites. Although the slopes of the lines appear to differ, this difference is not statistically significant ($F = 1.77$ } $F_{1,21} = 4.30$). The inaccuracy of our age estimates for this species resulted in a large error term. Therefore, if in fact a difference in growth rates did exist, we would have difficulty detecting it.

A. implicata appeared to grow more rapidly than *E. complanta* at both sites (slopes of .80 and 1.42 vs. .27 and .35, respectively), achieving a larger size in a shorter period of time. *A. undulata* was a much smaller organism, with a growth rate at Powder Mill Beach (slope = .52) more similar to *E. complanta* (.35) than *A. implicata* (1.42) from the same site. *A. implicata* and *A. undulata* did not seem to live as long as *E. complanta*. The oldest specimens collected were 17 years for *E. complanta*, 9 years for *A. implicata* and 7-8 years for *A. undulata*.

DISCUSSION

The three species of resident mussels from the Shubenacadie Lakes could be aged with varying degrees of accuracy. Age estimates were based on counts of internal growth bands, and were therefore somewhat

subjective. The agreement, or correlation, between counts made by two independent evaluators provided a measure of accuracy. Agreement was very good for E. complanta, but poor for A. implicata and A. undulata. Therefore, we have the most confidence in our age estimates for E. complanta.

It is impractical to section large numbers of shells for internal band counts. Instead, a model may be generated for estimating age from a more easily measured parameter which is a good predictor of age. We found valve length to be the best predictor of age for E. complanta and A. implicata, but shell weight was slightly better for A. undulata. For comparative purposes, valve length was used throughout. For all three species, a linear model for the valve length vs. age relationship was sufficient within the range of the data. This is certainly not true in general, and is due to the limited range of size and age classes sampled. If younger specimens (less than five years) in particular had been included, there may have been a significant curvilinear portion to the model.

Both evaluators counted more external than internal rings in A. implicata, suggesting that disturbance rings were being included in the external counts. This could lead to an overestimate of the true age for this species, therefore external counts should not be relied upon where accurate age estimates are required. In retrospect, internal band/external ring comparisons should have been made for E. complanta instead.

The main reason for aging our specimens was to determine the influence of age on interspecific differences in the bioaccumulation of As and Hg by mussels. Unfortunately, the age estimates for *A. implicata* and *A. undulata* were too inaccurate for this purpose.

E. complanata was the best species to work with. Growth rings were the clearest, age estimates were most accurate, and the prediction model had the best fit. As a result, we were able to detect differences in the growth of this species between the two sites. The Powder Mill Beach environment was much more contaminated with As and Hg, and the mussels here were significantly smaller. This suggests the possibility of sublethal toxic effects. We found *E. complanta* to be a poor regulator of mercury. Soft tissue concentrations increase almost exponentially with increasing body weight and age (Metcalf and Mudroch, 1985). In contrast, *A. implicata* of all sizes and ages maintain consistently low concentrations of this metal. It is therefore more likely that body burdens of Hg would accumulate to toxic levels in the tissues of *E. complanta*.

E. complanta has excellent potential as a biomonitoring species for these and other environmental contaminants in the Atlantic region. Because this mussel is long-lived, any contaminant which is poorly regulated will bioaccumulate over time - probably to detectable levels. Furthermore, a record of input over time can be obtained by analyzing various year classes. This species has been used extensively in environmental monitoring in Ontario and Quebec (Curry, 1977/78; Tessier et al, 1984; Kauss and Hamdy, 1985; A. Hayton,

Ontario Ministry of the Environment, personal communication). It occurs in the southern James Bay drainages and the St. Lawrence system (except Lake Huron south of Georgian Bay, Lake Michigan and most of Lake Erie), and the Atlantic drainage (Clarke, 1981). In the U.S.A., it is "... the most widespread of the East Coast mussels occurring in both polluted and unpolluted waters and lotic and lentic habitats from Maine to Florida ..." (Imlay, 1982). *A. implicata* and *A. undulata* are more restricted in their distributions, with the former occurring only in the Atlantic coastal drainage from Cape Breton to Maryland, and the latter found in the St. Lawrence River and its tributaries and the Atlantic drainage from Nova Scotia to Florida (Clarke, 1981). Therefore, from ecological, analytical and geographic points of view, *E. complanta* is the best choice for biomonitoring.

PART B: CONTAMINANT ANALYSIS OF MUSSEL SHELLS

INTRODUCTION

Recent studies suggest that the analysis of bivalve shells may offer several advantages over the analysis of body tissues when monitoring contaminant levels in the environment (Imlay, 1982; Fang and Shen, 1984). Shells can retain a record of contaminant levels over many years, and may provide a more sensitive and more permanent system for monitoring changes in ambient heavy metals (Fang and Shen,

1984). Imlay (1982) noted that for many trace elements the concentration factor is many times greater in the shell than in body tissues. Koide et al (1982) found that metal concentrations in the shells of marine mussels were more strongly correlated than concentrations in the soft tissues with proximity to highly industrialized or populated areas.

While analysis of whole shell is useful, more information could be gained by analyzing the annual growth rings separately. In this way changes in environmental contamination could be "mapped" over long periods of time. The methodology for this type of analysis is not currently available, however. Electron microprobe and secondary ion mass spectrometry (SIMS) appear to have good potential for this application. Thin sections prepared for aging may also be used for studying differences in the levels of trace elements between various years' growth. The electron microprobe and SIMS both measure elemental composition of prepared thin sections.

The electron microprobe is an x-ray instrument used to determine quantitatively the chemical composition of thin sections by means of electron bombardment and measurement of the subsequent x-rays produced. A curved crystal spectrometer is used to determine wavelength and intensity of the x-rays being emitted from the specimen. Comparisons are made of the intensities of x-rays from a material of known composition. Sensitivity of the microprobe is about 200 ppm (B. Barnett, Geology Department, University of Western Ontario, pers. comm.).

SIMS, a method similar to the electron microprobe, utilizes an ion beam which is focused onto the surface of a prepared sample. The secondary ions emitted are separated by mass for elemental discrimination and quantification. SIMS is a very sensitive technique, with a detection limit as low as 1 ppb for some elements.

This report evaluates the electron microprobe and SIMS procedures for determining arsenic, mercury and other trace element concentrations in mussel shells from the Shubenacadie Lakes.

MATERIALS AND METHODS

Four mussels were selected for study because of their high soft tissue concentrations of As, Hg and Pb (Table III). Shells were prepared and sectioned in the same manner as for aging, then polished and coated with carbon. Two specimens (LTE43 and PMA61) were chosen for microprobe analysis. Following the methods of Lutz (1981), who used the electron microprobe to measure strontium in Mytilus edulis shells, step scan analyses of 10 μm spots were made across the inner nacreous layer and down the surface periostracum as illustrated in Fig. 3. The electron beam was focused onto the thin section, inducing the emission of characteristic x-rays which were then separated in order to discriminate among As, Hg, Pb, Cd, Ca, Sb and Zn.

Specimens PME21 and PMA63 were chosen for the more sensitive SIMS analysis. Step scan analyses of 25 μm spots were made across the nacreous layer and down the periostracum, as described for the

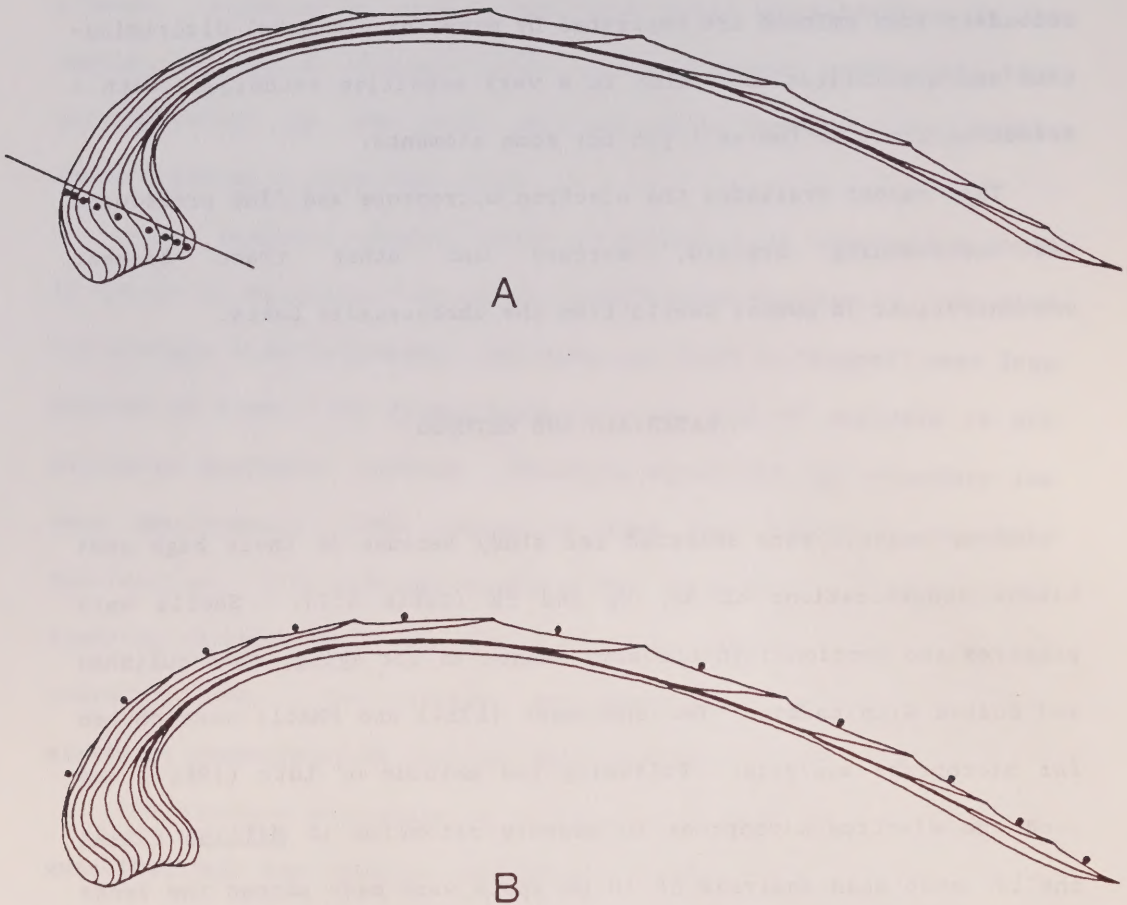


Figure 3. LOCATION OF SPOTS FOR STEP SCAN ANALYSIS ACROSS THE INNER NACREOUS LAYER (A) AND DOWN THE SURFACE PERIOSTRACUM (B)

TABLE III. Soft tissue concentrations of As, Hg and Pb (ug/g dry weight) in four mussels selected for shell contaminant analysis.

Mussel	Shell Analysis Procedure	Soft Tissue Concentration (ug/g dry weight)		
		As	Hg	Pb
Lake Thomas, E. <u>complanata</u> , 10 years old (LTE43)	Electron microprobe	30.5	4.6	29.0
Powder Mill Beach, A. <u>implicata</u> , 9 years old (PMA61)*	Electron microprobe	45.0	.8	6.5
Powder Mill Beach, E. <u>complanata</u> , 10 years old (PME21)	SIMS	42.0	1.2	NA
Powder Mill Beach, A. <u>implicata</u> , 6 years old (PMA63)*	SIMS	45.0	.8	6.5

NA = not analyzed

* Soft tissues of these specimens were combined for analysis

microprobe method. A survey spectrum was run to measure all elements with masses ranging from 0 to 240 mass units. To examine elements present at low concentrations, extended counting techniques (30s/mass vs. 0.5s/mass) were employed on masses of particular interest (As, Hg, Pb, Cd). The measured ion intensity ratios were converted to concentrations using an ion yield curve determined from a lead silicate glass standard.

RESULTS AND DISCUSSION

Results of the electron microprobe analysis were extremely variable (Table IV), therefore levels of As, Hg, Cd and Zn in the shells could not be quantified. Reliable estimates were not possible due to the instrument's maximum sensitivity of 200 ppm, and its inherent variability at the low concentrations encountered. The electron microprobe is intended for measuring elements in percent concentrations in geological materials. Lutz (1981) reached similar conclusions when he attempted to study seasonal variation in strontium concentration in Mytilus edulis.

Preliminary spectral surveys with SIMS detected only major shell elements in the parts per thousand and percent concentration range (i.e. Ca, C, Sr, Na, Fe, Mn). Subsequent analyses involved counting ion intensity for extended periods on masses of particular interest (i.e. As, Hg, Pb, Cd). Even under these conditions, these elements could not be quantified due to extreme variability (Table V).

TABLE IV. Concentrations of various elements in the shell of mussel LTE43, as determined by the electron microprobe.

Element	Concentration as ug/g dry weight (mean $\pm$ s.d.)*
As	190 $\pm$ 200
Hg	1100 $\pm$ 1600
Pb	ND
Cd	ND
Sb	1600 $\pm$ 500
Zn	190 $\pm$ 600

* n = 20 spots (14 across the inner nacreous layer and 6 down the surface periostracum).

TABLE V. Concentrations (as ug/g dry weight) of various elements in the shells of mussels PMA63 and PME21, as determined by SIMS.

Element	PMA63	PME21	
		Spot #1	Spot #2
Al	15,800 $\pm$ 14,000	2600 $\pm$ 1800	7300 $\pm$ 3700
V	2000 $\pm$ 300	2500 $\pm$ 600	3000 $\pm$ 1200
Fe	4700 $\pm$ 3400	2400 $\pm$ 300	6200 $\pm$ 800
Cu	90 $\pm$ 30	80 $\pm$ 60	120 $\pm$ 40
As	70 $\pm$ 60	ND	30 $\pm$ 60
Sr	540 $\pm$ 30	570 $\pm$ 140	650 $\pm$ 200
Pb	90 $\pm$ 99	100 $\pm$ 170	ND
Cd	ND	ND	ND
Hg	ND	ND	ND

ND = not detectable

* mean $\pm$ s.d. for n = 3 readings

A number of modifications could be made to the technique to improve sensitivity. First, a more appropriate calibration standard could be used. Lodding et al (1984) suggested using an apatite crystal when utilizing SIMS in the quantitative analysis of tooth and bone hard tissue. Second, surface charge-up should be controlled by the use of specially adapted metal sample holders or the application of aluminum grids on the sample surfaces (Lefevre, 1980). Finally, Lodding et al (1984) believed that a better understanding of the complicated spectra of hard biological tissues and methods of suppressing intrinsic molecular peaks would greatly aid in the quantitative analysis of hard biological tissues.

Although many authors have suggested that bivalve shells offer several advantages over soft tissues for monitoring pollution events, there remain unsolved problems in shell analysis. Little is known about how contaminants bond within the calcium carbonate matrix. Swann et al (1984) observed changes in Zn levels within the same annual growth increment, and suggested that elemental adsorption onto the shell was occurring. In the same experiment Mg, Al, Si, Sg, Mn and Fe content decreased over time, suggesting that elemental leaching could be occurring. Fang and Shen (1984) also warned that chemicals bound in the old parts of the shell will begin to leach out into the water. They also observed that pollutants tend to accumulate on the outer shell surface where leaching is most likely to occur. Koide et al (1982) believed that microorganisms on the shell surface may account for high surface metal concentrations.

The two techniques, electron microprobe and SIMS, failed to detect quantifiable levels of As, Hg, Pb or Cd in the shells of mussels known to be highly contaminated with As and Hg. The electron microprobe, with its maximum sensitivity of about 200 ppm and high variability near its limits of detection, was not suitable for measuring trace elements at the levels found in these shells. The more sensitive SIMS technique detected only major shell elements. With further method development, including the reduction of interferences and the production of specially prepared standards and appropriately designed sample holders, this time consuming and expensive method may prove useful in the analysis of contaminants within and between annual growth increments. At that time, mussel shells may become invaluable in the study of both past and present pollution events.

SUMMARY

Freshwater mussels from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg). Soft tissue concentrations of these elements vary among species and lakes, and are influenced by body weight. Age is also suspected to be a factor. To determine the relationship between age and the bioaccumulation of As and Hg, and to compare longevities and growth rates among species and lakes, the shells of three species of mussels from two lakes (n=249) were aged. Fifteen Elliptio complanata, 93

Anodonta implicata and 30 Alasmidonta undulata were collected from the most contaminated site, Powder Mill Beach, while 67 E. complanata and 44 A. implicata were collected from a less contaminated site, Lake Thomas. Ten to 13 representative specimens of each species/site combination were aged directly, that is, shells were thin-sectioned and annual internal growth bands were counted independently by two evaluators. External growth rings were also counted for A. implicata. Ages of the remaining specimens were estimated using a regression model based on valve length as a predictor of age.

E. complanata could be aged with much more accuracy than either A. implicata or A. undulata. Internal growth bands were clearest, agreement between evaluators was closest ($r = .835$ vs. $.586$ and $.536$, respectively), and the regression model had the best fit ($r = .88$ vs. $.70$ and $.80$, respectively). External ring counts overestimated the ages of A. implicata, and should not be used. A. implicata grew more rapidly ($.80 - 1.42$ cm/yr.) than E. complanata ($.27 - .35$ cm/yr.), achieving the same maximum valve length (≈ 11.5 cm) in less time ($8.5 - 10.5$ vs. $17 - 20$ yrs.). A. undulata was smaller, with a growth rate at Powder Mill Beach similar to E. complanata ($.52$ vs. $.35$ cm/yr.). E. complanata appeared to live longer (>17 yrs.) than A. implicata (>9 yrs.) or A. undulata (>8 yrs.). E. complanata from Powder Mill Beach were significantly smaller at a given age than those from Lake Thomas. Because the growth rates of specimens aged 5-17 yrs. did not differ, E. complanata must have grown more slowly at Powder Mill Beach during the first five years of life. As this site is the most

contaminated with As and Hg, the possibility of sublethal toxicity is suggested.

The influence of age on interspecific differences in the bioaccumulation of As and Hg by mussels could not be determined, because age estimates for both *A. implicata* and *A. undulata* were too inaccurate. However, the data for *E. complanata* suggest that it would be the best choice for biomonitoring purposes in the Atlantic region. Because it is long-lived, contaminants which are poorly regulated should accumulate over time to detectable levels. Also, because it can be accurately aged, various year classes can be analyzed separately. This species is widely distributed throughout central and eastern Canada, and has been used extensively for biomonitoring in Ontario and Quebec. Therefore, there is an existing data base for comparing environmental contamination in the Atlantic region with other regions in Canada.

Mussels accumulate metals in their shells as well as their soft tissues. Shells retain a sequential record of contamination in their annual growth rings, and therefore have potential for mapping changes in environmental contamination over time. Several shells were subjected to trace element analysis of their growth rings by two techniques, the electron microprobe and secondary ion mass spectrometry (SIMS). Both techniques failed to detect measureable concentrations of As, Hg, Pb or Cd in the shells. The microprobe, with a detection limit of approximately 200 ppm, was not sensitive enough. SIMS has a theoretical detection limit as low as 1 ppb, but

factors such as mass spectral interferences and high counting times may have decreased its sensitivity. This method requires further development and refinement before it can be used for this application.

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APPENDIX IA. Valve lengths, shell weights, and age estimates from internal bands for *E. complanata* which were sectioned.

Site	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages	
				Evaluator #1	Evaluator #2
Lake Thomas	1	7.30	13.678	5	5
	3a	7.90	17.173	8	8
	4a	8.20	22.079	14	8
	5a	8.75	23.947	9	8
	6a	9.10	28.367	13	12
	7a	9.30	32.586	8	9
	8b	9.60	29.813	7	8
	9b	9.90	34.941	13	13
	11c	10.20	37.135	20	14
	12b	10.50	64.100	11	13
	14b	11.50	37.300	12	12
	14c	11.55	56.830	17	16
Powder Mill Beach	10	4.80	3.204	5	6
	9	4.90	3.551	4	6
	6	7.30	17.865	6	7
	8	7.35	19.554	9	11
	3	7.45	15.486	7	8
	7	7.70	22.270	11	13
	2	7.80	24.166	10	9
	5	8.20	19.255	12	13
	4	8.30	24.768	12	14
	1	8.40	26.601	13	13

APPENDIX IB. Valve lengths, shell weights, and age estimates from the regression model for E. complanata which were not sectioned.

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Lake Thomas	85	2a	7.55	19.348	4.5
		2b	7.60	20.278	4.5
		3c	7.65	13.092	5.0
		3b	8.10	23.431	6.5
		17b	8.15	22.650	6.5
		4b	8.20	24.385	6.5
		17c	8.20	32.484	6.5
		4c	8.50	25.796	7.5
		17a	8.55	28.239	8.0
		5b	8.75	26.944	8.5
		6c	8.95	22.714	9.0
		16a	9.00	21.462	9.5
		16b	9.00	31.705	9.5
		16c	9.00	23.109	9.5
		6b	9.15	28.115	10.0
		7b	9.25	31.473	10.0
		7c	9.25	36.165	10.0
		18a	9.45	35.564	11.0
		8a	9.50	32.168	11.0
		8e	9.50	27.835	11.0
		8c	9.55	31.122	11.0
		18b	9.55	31.864	11.0
		18c	9.55	39.153	11.0
		9a	9.60	25.073	11.5
		8d	9.65	45.130	11.5
		8f	9.65	34.590	11.5
		9c	9.75	41.590	12.0
		5a	9.80	21.605	12.0
		10a	10.05	33.139	13.0
		10b	10.05	48.660	13.0
		11b	10.15	31.830	13.0
		10c	10.20	53.950	13.5
		11a	10.20	49.610	13.5
		19a	10.20	78.810	13.5
		19b	10.20	43.570	13.5
		19c	10.30	48.120	13.5
		12c	10.50	37.455	14.5
		12a	10.60	67.390	14.5
		13	10.70	41.200	15.0
		14a	11.00	62.210	16.0
		15b	11.15	28.155	16.5
		15a	11.30	43.660	17.0

APPENDIX IB. Valve lengths, shell weights, and age estimates from the regression model for E. complanata which were sectioned.

continued

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Lake Thomas	84	21	7.90	27.849	5.5
		22	8.4	20.285	7.5
		24	8.4	21.166	7.5
		23	8.5	18.760	7.5
		25	8.8	31.239	8.5
		34	10.0	49.22	12.5
		32	10.2	43.15	13.5
		44	10.2	83.75	13.5
		31	10.5	43.78	14.5
		33	10.7	50.31	15.0
		43	11.0	61.73	16.0
		42	11.2	51.46	16.5
		41	11.5	61.70	17.5
Powder Mill Beach	84	25	6.7	10.478	7.5
		24	7.2	14.341	9.5
		23	7.4	15.442	10.0
		22	7.6	12.069	10.5
		21	7.6	15.625	10.5

APPENDIX IIA. Valve lengths, shell weights, age estimates from internal bands, and age estimates from external rings for A. implicata which were sectioned.

Site	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages			
				Internal Bands		External Rings	
				Eval. #1	Eval. #2	Eval. #1	Eval. #2
Lake Thomas	11b	5.20	1.740	5	5	5	5
	11a	5.25	2.101	5	5	5	4
	10a	6.00	2.598	5	4	7	6
	10b	6.35	4.449	6	5	7	5
	9c	6.70	4.713	7	6	6	6
	4	7.10	4.953	5	4	6	5
	5c	7.80	6.479	7	6	7	5
	6	8.00	10.650	9	6	8	7
	7c	8.30	11.772	11	7	10	8
	3	8.90	12.868	7	7	9	8
	2	9.70	12.553	6	5	11	10
	1	11.70	33.330	9	6	11	10
Powder	2b	4.15	0.976	4	4	4	3
Mill Beach	2c	4.60	1.089	5	4	5	3
	3b	5.30	1.819	5	4	5	3
	4b	6.00	2.421	7	5	8	4
	5a	6.70	4.855	6	4	6	5
	6a	6.80	7.381	7	6	7	6
	7c	7.20	5.364	6	4	7	7
	9a	7.80	10.043	7	6	6	7
	10a	8.60	17.184	6	5	6	5
	12b	9.10	22.904	6	9	7	9
	16f	9.90	26.619	8	8	9	10
	17	10.35	24.664	6	6	7	8
	19b	10.70	29.835	8	8	9	10

APPENDIX IIB. Valve lengths, shell weights, and age estimates from the regression model for *A. implicata* which were not sectioned.

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Lake Thomas	85	11c	4.35	0.803	3.0
		14a	5.70	2.131	4.5
		14b	5.75	2.948	4.5
		14c	5.90	1.916	4.5
		10c	6.30	3.742	5.0
		13	6.40	3.748	5.0
		9a	6.65	5.860	5.0
		8	6.80	4.699	5.5
		9b	6.80	4.244	5.5
		5a	7.30	4.288	6.0
		5b	7.35	5.489	6.0
		12	7.40	5.357	6.0
		7a	8.30	6.154	6.5
		7b	8.45	10.650	7.0
	84	26	4.60	1.369	3.5
		24	5.1	2.031	3.5
		25	5.1	1.975	3.5
		22	5.2	2.276	4.0
		23	5.6	2.759	4.0
		21	5.7	2.484	4.5
		34	6.4	3.685	5.0
		31	6.7	3.962	5.0
		32	6.8	5.007	5.5
		33	7.1	4.003	5.5
		43	7.4	8.236	6.0
		42	7.7	7.259	6.0
		44	8.3	10.594	6.5
		51	8.3	9.508	6.5
Powder Mill Beach	85	41	8.4	14.263	7.0
		52	8.4	11.601	7.0
		53	9.5	13.719	8.0
		54	9.8	17.738	8.0
		2a	4.50	1.241	3.0
		21c	4.80	1.332	3.5
		3a	5.45	2.544	4.0
		21a	5.50	1.874	4.0
		4a	5.80	2.475	4.5
		4c	6.25	3.818	5.0
		5c	6.65	4.508	5.0
		23b	6.70	4.734	5.0
		23a	6.75	6.128	5.5
		23c	6.75	6.404	5.5
		5b	6.80	5.161	5.5
		6c	6.90	6.158	5.5
		6b	7.10	8.873	5.5
		7b	7.25	5.902	5.5
		7a	7.30	9.171	6.0

APPENDIX IIB. Valve lengths, shell weights, and age estimates from the regression model for A. implicata which were not sectioned.

continued

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Powder Mill Beach	85	24b	7.30	6.984	6.0
		8d	7.40	8.929	6.0
		8a	7.50	7.029	6.0
		8c	7.50	6.302	6.0
		24c	7.55	7.465	6.0
		24a	7.60	5.538	6.0
		8b	7.60	5.941	6.0
		10b	7.70	10.671	6.0
		9d	7.85	6.726	6.5
		9b	7.90	9.205	6.5
		10d	8.20	10.488	6.5
		10c	8.40	15.885	7.0
		11b	8.40	15.432	7.0
		22b	8.70	13.888	7.0
		22c	8.75	12.962	7.0
		11a	8.75	16.387	7.0
		11c	8.80	14.938	7.0
		22a	8.80	13.725	7.0
		9c	8.90	7.786	7.5
		12c	8.90	20.577	7.5
		12a	8.95	20.752	7.5
		13b	9.15	15.322	7.5
		13c	9.25	16.716	7.5
		13a	9.40	17.621	7.5
		14c	9.40	17.456	7.5
		14b	9.45	20.786	8.0
		15b	9.55	24.195	8.0
		14a	9.60	12.582	8.0
		15c	9.70	26.591	8.0
		15a	9.75	18.691	8.0
		25b	9.85	21.460	8.0
		25a	9.90	18.884	8.0
		16b	9.90	20.859	8.0
		16d	10.00	26.550	8.5
		16e	10.00	23.062	8.5
		25c	10.10	22.511	8.5
		16c	10.20	20.810	8.5
		16a	10.35	26.896	8.5
		20a	10.40	27.422	8.5
		18	10.50	28.224	9.0
		19a	10.50	24.783	9.0
		20b	10.75	33.685	9.0
		20c	10.90	30.552	9.0
		3c	NM	1.281	-
		21b	NM	1.367	-

APPENDIX IIB. Valve lengths, shell weights, and age estimates from the regression model for A. implicata which were not sectioned.
continued

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Powder Mill Beach	84	36	3.9	0.875	2.5
		33	4.3	1.165	3.0
		34	4.3	1.034	3.0
		35	4.3	1.053	3.0
		32	4.4	1.460	3.0
		31	4.7	1.691	3.5
		46	6.3	4.505	5.0
		44	6.6	4.190	5.0
		45	6.7	6.846	5.0
		43	6.8	4.618	5.5
		42	7.0	5.395	5.5
		41	7.2	7.649	5.5
		51	9.2	17.468	7.5
		52	9.2	22.698	7.5
		53	9.7	16.877	8.0
		54	10.0	20.414	8.5
		64	10.2	35.673	8.5
		61	10.3	37.699	8.5
		63	11.2	45.01	-
		62	11.3	41.55	9.5

NM = not measured

APPENDIX IIIA. Valve lengths, shell weights, and age estimates from internal bands for *A. undulata* which were sectioned.

Site	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages	
				Evaluator #1	Evaluator #2
Powder Mill Beach	20	2.90	1.069	6	3
	19	3.05	1.336	5	4
	18	3.15	1.555	7	4
	17	3.20	1.618	6	4
	16	3.30	1.914	6	4
	14	3.40	2.242	5	4
	12	3.80	3.712	8	5
	7	4.00	3.503	7	5
	6	4.20	4.679	8	4
	4	4.40	4.818	5	5
	2	4.50	5.315	8	6
	1	4.90	7.061	8	7

APPENDIX IIIB. Valve lengths, shell weights, and age estimates from internal bands for *A. undulata* which were not sectioned.

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Powder Mill Beach	85	15	3.45	1.674	5.0
		11	3.80	3.554	5.5
		13	3.90	2.845	6.0
		8	4.00	4.031	6.0
		9	4.00	3.677	6.0
		10	4.00	3.362	6.0
		5	4.30	3.995	6.5
		3	4.70	5.517	7.5
	84	25	2.5	0.673	3.0
		23	3.0	1.510	4.0
		21	3.2	1.996	4.5
		24	3.3	1.986	4.5
		22	3.4	2.054	5.0
		34	3.4	2.692	5.0
		35	3.4	2.051	5.0
		33	3.5	2.505	5.0
		32	3.8	3.051	5.5
		31	4.0	3.843	6.0

APPENDIX 2

SEMINARS PRESENTED ON M.O.E. FUNDED RESEARCH

- 1) Bailey, R.C. 1985. Variation in molluscan communities along an environmental gradient. Canadian Congress of Biology, London, Ontario.
- 2) Bailey, R.C. 1986. Substrate preference in a freshwater mussel. Abstract from 34th North American Benthological Society (NABS) in Lawrence, Kansas.
- 3) Bailey, R.C. 1986. Relationship between exposure and the shell growth and form of a freshwater mussel. 71st Annual meeting of the Ecological Society of America Syracuse, N.Y.
- 4) Bailey, R.C. 1987. Variation in shell morphology and growth rate of the unionid bivalve Lampsilis radiata: Nature, nurture or nurtured nature? NABS mtg. in Orono, Maine.
- 5) Bailey, R.C. 1987. Variation in shell morphology and growth rate of the unionid bivalve Lampsilis radiata: Nature, nurture or nurtured nature? Abstract from Canadian Paleontology & Biostratigraphy Seminar held in London, Ontario September 1987.
- * 6) Green, R.H. 1987. Use of bivalve molluscs as environmental monitors. Invited speaker at Mount Allison University, Sackville, New Brunswick.
- 7) Green, R.H. 1987. Shell form and exposure to wave energy in the unionid bivalve Elliptio complanata. Abstract from Canadian Paleontology & Biostratigraphy Seminar held in London, Ontario September 1987.
- 8) Hinch, S.G. 1986. The effects of exposure and alkalinity on morphometry of the freshwater unionid clam Elliptio complanata. Abstract from NABS mtg. in Lawrence, Kansas.
- 9) Hinch, S.G. 1987. Within and among lake variation in shell morphology of a freshwater clam from south central Ontario Lakes. Abstract from Canadian Paleontology and Biostratigraphy Seminar, held in London, Ontario September 1987.
- 10) Hinch, S.G., Bailey, R.C. and R.H. Green. 1985. The effects of environmental change on the growth of a freshwater unionid clam, Lampsilis radiata. Can. Congress of Biology.
- 11) Hinch, S.G. and R.H. Green. 1987. Shell etching: a chemical or physical process? 35th Ann. Mtg. of NABS, in Orono, Maine.
- 12) Walker, C. 1987. Analysis for contaminants in bivalve mollusc shells: a useful approach for pollution studies? Abstract from Canadian Paleontology and Biostratigraphy Seminar, held in London Ontario September 1987.

* No abstract available.

2)

102

SUBSTRATE PREFERENCE IN A FRESHWATER MUSSEL. Robert C. Bailey. Dept. of Zoology, University of Western Ontario, London, Ontario. N6A 5B7.

I tested experimentally the hypothesis that freshwater mussels can exhibit substrate preference. One hundred Lampsilis radiata from each of two habitats (sand, silt) were collected in Inner Long Point Bay, Lake Erie. Fifty mussels from each habitat were placed in each of two artificial ponds (6m X 9m). The initial position of each mussel on the bottom of the ponds was determined using a random number generator. Equal areas of two substrate types (sand, silt) were present in each pond. After four months the mussels were retrieved. Those which had been collected from a sandy natural habitat preferred a silty substrate in the ponds (3:1), while mussels initially collected from a silty natural habitat showed no substrate preference in the ponds. This did not indicate a lack of movement in mussels from the silt, since the same proportion of animals in each group (34%) changed position during the study. The preference for silt by formerly sand-dwelling mussels showed that substrate selection was not simply based on particle size. Since mussels from different natural habitats exhibited different degrees of substrate preference, selection for this trait may vary in nature.

103

POPULATION REGULATION OF THE LOTIC GASTROPOD ELIMIA CAHAWBENSIS (PLEUROCERIDAE) BY TREMATODES. Terry D. Richardson and Joseph F. Scheiring. Department of Biology, University of Alabama, Tuscaloosa, AL 35486.

The effect of transient parasites upon the population regulation of their intermediate hosts has received less attention than that of parasitoids even though numerous animals serve as intermediate hosts. This is primarily because parasites do not kill their hosts and apparently cause only a slight reduction of individual host fitness. In this study we suggest that these parasites may play a major role in the population regulation of the freshwater gastropod Elimia cahawbensis (Anthony) in Little Schultz Creek, Bibb County, Alabama. Random samples spanning the peak reproductive season of the population indicated that on the average 36.6% of the females were infected with trematode parasites. In addition, it was found that 95.8% of the infected females that had reached the age of first reproduction were reproductively inactive. This represented a significant reduction ($P < .01$) in the egg laying potential of the population. Although these individuals are capable of continued intraspecific interactions, they have essentially been removed from the population and do not contribute to its increase. Therefore, we suggest that the infection by trematodes with the concomitant reduction in reproductive potential may contribute significantly to the natural population regulation of this freshwater gastropod.

104

THE REPRODUCTIVE BIOLOGY OF FOUR FRESHWATER MUSSELS IN THE NEW RIVER IN VIRGINIA/WEST VIRGINIA. Kurt J. Jirka and Richard J. Neves. Virginia Cooperative Fisheries and Wildlife Research Unit, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

The annual reproductive cycles of four freshwater mussels, Actinonaias carinata, Elliptio dilatata, Cyclonaias tuberculata, and Tritogonia verrucosa, from the New River were determined from histological sectioning of gonadal tissue. Six individuals of each species were collected monthly from August 1984 to February 1985, and approximately every two weeks from March to July 1985. Examination of stained tissue sections indicated that A. carinata is bradyctictic, with spawning occurring in mid-summer. Females became gravid by September, and glochidia overwintered within the female, being released in late spring. The other three species are tachyctictic. Spawning in C. tuberculata occurred from January through April; gravid females were present from April through June. Spawning in T. verrucosa and E. dilatata occurred from March through April; gravid females of T. verrucosa were present from April through June, and E. dilatata females were gravid from April to early July. It appears that glochidial release for these three species occurs throughout the spring and early summer months, since all stages of developing glochidia were found in the female marsupia during this period. All four species are dioecious, but some hermaphroditic specimens of A. carinata and E. dilatata were observed.

4)

137

VARIATION IN THE SHELL MORPHOLOGY AND GROWTH RATE OF A FRESHWATER MUSSEL : NATURE, NURTURE, OR NURTURED NATURE? Robert C. Bailey. Department of Zoology, University of Western Ontario, London, Ontario, Canada. N6A 5B7.

Correlations between the shell morphology and growth rate of freshwater mussels and their environment have been described for over 70 years (e.g. Ortmann's 'Law'). I measured such a correlation in Lampsilis radiata siliquioidea from Inner Long Point Bay, Lake Erie. I the used allozyme data, heritability estimates, and a substrate preference experiment to form a hypothesis for the genetic mechanism causing the character variation. I also carried out burrowing experiments to test the adaptive hypotheses previously proposed for variation in shell morphology. Evidence from all of these studies suggests the presence of adaptive plasticity in these mussels.

138

POPULATION GENETIC STRUCTURE AND LIFE HISTORY CHARACTERISTICS OF A MAYFLY SIPHOPLECTON BASALE IN EASTERN NORTH AMERICA. Bernard W. Sweeney, Robin L. Vannote, and David H. Funk. Stroud Water Research Center, Academy of Natural Sciences, Avondale, PA

S. basale occurs in rivers throughout Canada and the eastern portion of the United States. The species was univoltine throughout our study range (i.e. Quebec, Maine, Vermont, Delaware, Virginia, and North Carolina). Adult emergence dates ranged from early March (North Carolina) to mid-June (Quebec). Adult biomass was greatest in Vermont (e.g. 21 mg) and decreased at more southern (e.g. 12 - 16 mg; North Carolina) and northern (e.g. 6 - 14 mg; Quebec) locations. Variation in adult biomass reflects differences in environmental quality during the early larval stages.

Population genetic structure was assessed using protein electrophoresis. Twenty-three loci were examined for about 40 individuals in each population. The frequency of polymorphic loci (30%) and level of heterozygosity (0.11) was higher than most other mayflies. No fixed allelic differences were observed among populations but allele frequencies varied significantly among sites. Geographic differentiation of population structure was higher than for most mayfly species (Wright's F_{ST} averaged 0.21) but no geographic trend was observed. Estimates of gene flow between populations were low (Slatkin's $N_m = 0.52$).

The S. basale population in Eastern North America seems to be highly segregated, with limited gene flow between subpopulations. Genetic differentiation seems random and probably reflects genetic drift among isolated subpopulations. Life history variation seems to be largely ecophenotypic, although a genetic component cannot be absolutely ruled out at the present time.

139

TIMING OF ADULT EMERGENCE OF UNIVOLTINE EPHEMEROPTERA: A PROPOSED MODEL AND ITS IMPLICATIONS FOR GEOGRAPHIC RANGE AND ADULT SIZE VARIATION. J. Denis Newbold, Bernard W. Sweeney, and Robin L. Vannote. Stroud Water Research Center, Academy of Natural Sciences, Avondale, PA

Univoltinism and the timing of adult emergence of hemimetabolous aquatic insects are generally explained in terms of photoperiod effects and diapause mechanisms. We show, however, that a simple two-stage life history model consisting of two different thermal responses can explain univoltinism and emergence timing for a number of mayfly species. First-stage development normally occupies 60-85% of the year and proceeds independently of temperature or at a rate inversely related to temperature. In the second stage, development rate increases with temperature. Applied to 14 species, this model explains an average of 80% of the variance in emergence date over the geographic range, and predicts emergence date at a given site with an average error of ± 9 days. For several species, the model implies that average temperature during 2-4 months prior to emergence is nearly uniform geographically, regardless of annual average temperature, and this uniformity is observed in the field data. This uniformity has two further implications: (1) To the extent that geographic variation in adult size is attributable to thermal regime, such variation arises from thermal effects on growth (as opposed to development) during the first 8-10 months, typically prior to most of the biomass accumulation. (2) Geographic range may be limited by the absence of a 2 to 4-month period with the average temperature necessary to maintain a univoltine life history. We present evidence that this criterion may explain the southern geographic limits of several species.

140

A TEST OF THE THERMAL EQUILIBRIUM HYPOTHESIS FOR INSECTS IN WARMWATER STREAMS. Emily Stanley. Dept. of Zoology, Arizona State University, Tempe, AZ. 85282 and Robert Short. Aquatic Station, Southwest Texas State University, San Marcos, TX. 78666.

The predictions of the Thermal Equilibrium Hypothesis (Vannote and Sweeney 1980) were tested in warmwater streams with both constant and fluctuating thermal regimes for various mayfly species. Insects were collected for one year, identified, counted, and placed into size classes on the basis of head capsule measurements. Representative individuals from each size class were dried and weighed to determine population biomass on each sampling date. Mature females from each month were also weighed to determine body size and fecundity throughout the study period. Fecundity varied in a predictable fashion for Baetis quilleri and Traverella presidiana in a thermally fluctuating stream, with maxima occurring in the winter (0.506 and 3.265 mg respectively) and minima in the summer (0.115 and 0.924 mg). There was no apparent trend for B. quilleri or Baetodes edmundsi in a thermally constant stream. In both streams, there was no relationship between individual body size and population biomass. Further, there was either an inverse or no relationship between individual fecundity and population density, contrary to the predictions of the Thermal Equilibrium Hypothesis. It is proposed that "equilibrium" conditions for multivoltine warmwater insects may not only be a function of geographic location (Vannote and Sweeney 1980), but also of time and that maximum body size does not necessarily indicate thermally optimal conditions.

11)

SHELL ETCHING: A CHEMICAL OR PHYSICAL PROCESS ? Scott G. Hinch and Roger H. Green.
Dept. of Zoology, University of Western Ontario, London, Ontario, Canada. N6A 5B7.

Freshwater unionid clams are often missing portions of their protective shell coat (periostracum) revealing prismatic and nacreous shell layers. This etching occurs primarily around the umbo of the shell. It has long been thought that this phenomenon is a chemical process analogous to the weathering of calcareous rock by weak acids. In poorly buffered habitats affected by acid precipitation, this dissolution should be enhanced. We collected *Elliptio complanata* from an area of high exposure (high water turbulence) and low exposure (low water turbulence) in 4 lakes that lie on an alkalinity gradient. Shell etching was quantified using an image analysis process on a micro-computer based digitizing system. Clams from the high exposure site were more etched than those from the low ($P < 0.05$) in each lake after correcting for clam size differences. Over half of the total variation in shell etching resulted from differences between exposures (within-lake) while none of the total variation was due to differences between lakes. These results suggest that shell etching is a physical process. Shells probably become eroded by abrasive particles that are suspended in the water column. Shell erosion would not be a good predictor of lake acidification.

99

EFFECTS OF VOC CONTAMINATION ON THE STREAM FISH, PIMEPHALES PROMELAS (RAFINESQUE). Gary Welker, Paul Liechti, Don Huggins, and Tamara Frazier. Kansas Biological Survey Lawrence, KS.

The fathead minnow was utilized in a series of "in situ" and laboratory bioassay tests conducted to assess the impact of volatile organic compound intrusion on a small intermittent stream in SE Kansas. Spring water contaminated by VOC's originating from a hazardous waste site, uncontaminated spring water and various mixtures of contaminated and uncontaminated spring and receiving stream water were tested in the field and laboratory for both acute and chronic effects on fatheads. Juvenile minnows contained in plexiglass enclosures were exposed "in situ" for up to 96 h. Despite continuous exposure to VOC concentrations up to 308.6 ug/L, low dissolved oxygen, and other water quality extremes no juvenile deaths were recorded at any water quality site. The primary VOC constituents found during this study were 1,2-Dichloroethane, 1,2-Transdichloroethylene, and vinyl chloride. Chronic laboratory tests were performed by exposing the more sensitive larval fish stage (< 24 h old) to water samples from various water quality sites (e.g., springs, contaminated stream water). Initial tests revealed complete larval mortality at high VOC concentrations (> 172 ug/L) but no significant mortality differences between low concentrations (< 6 ug/L) and controls. Following this test the VOCs were purged from water samples with nitrogen gas, dissolved oxygen levels adjusted and the initial chronic test repeated. Test results for spring water purged of VOCs indicated that the previously observed fish mortality was due to the presence of VOCs in this water. Known chronic LC50 values for single compounds in the observed VOC mixture suggest no one compound should have caused the mortality noted in the initial chronic bioassay.

100

EFFECTS OF ACUTE ACID EXPOSURE ON THE CRAYFISH CAMBARUS BARTONII BARTONII. Robert J. DiStefano, Richard J. Neves, and Louis A. Helfrich, Virginia Cooperative Fishery Research Unit, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

Laboratory flow-through toxicity tests were conducted to examine acute effects of acidification and the influence of ambient water temperature on pH tolerance in populations of Cambarus bartonii bartonii from high-elevation southern Appalachian streams. Ninety-six hour LC50 values were determined for three size classes of crayfish. Tolerance to low pH (sulfuric acid) appeared to be positively correlated with increasing size of crayfish. LC50 values demonstrated an increased tolerance at reduced water temperature (15°C vs. 21°C). Examination of hemolymph cation concentrations and carapace Ca^{++} in crayfish held at low pH's indicated that hemolymph Na^{+} decreased when compared to controls after 96 hour exposure to the LC10. Levels of $[Ca^{++}]$, $[Mg^{++}]$ and $[K^{+}]$ in hemolymph from acid-exposed specimens were similar to those from control specimens. No significant changes were observed in exoskeleton (carapace) $[Ca^{++}]$ subsequent to a 4 day exposure (pH 2.46 - 2.96). The molting process apparently was inhibited, as exoskeletons failed to become rigid following placement of molting crayfish in extremely acidic water.

8)

101

THE EFFECTS OF EXPOSURE AND ALKALINITY ON MORPHOMETRY OF THE FRESHWATER UNIONID CLAM ELLIPTIO COMPLANATA. Scott G. Hinch. Dept. of Zoology, University of Western Ontario, London, Ontario, Canada. N6A 5B7.

I collected and measured similarly aged E. complanata from a low and high exposure area in each of four small lakes of varying alkalinity in south-central Ontario. Nested ANOVAs on shell length, height, width and weight revealed that much more of the variation in shell morphometry was due to within-lake (i.e., between exposures) rather than among-lake variation. A canonical variate analysis of between-exposure variation showed that clams from high exposure areas were larger and proportionately taller and heavier than those from low exposure areas. This study showed that the use of unionid shell morphometry as a predictor of whole lake characteristics such as alkalinity requires careful consideration of morphometric variation within each lake.

CZ9.02

UPTAKE OF ZINC BY RAINBOW TROUT: RELATIVE CONTRIBUTIONS OF DIETARY AND WATERBORNE INPUTS. D. J. Spry Department of Biology, McMaster University, Hamilton, Ontario, Canada. L8S 4K1. Fingerling rainbow trout, Salmo gairdneri, were exposed to combinations of zinc in water (0, 0.04, 0.14, 0.50 mg/L) and a semi-purified diet (0, 100, 700 mg/kg). Growth rate and plasma ions were measured at 0, 1, 2, 4, 8 and 16 w. At 16 w, fish receiving low zinc from both sources were zinc deficient, based upon poor growth (1.7 fold increase) and severely depressed plasma zinc (0.008 ± 0.005 (9) mequiv/L). Increased levels of zinc from either source repeated zinc in the plasma (0.401 ± 0.051 mequiv/L) and increased growth rate (4 fold increase). This suggested that zinc may be taken up by either of two routes: intestinal or extra-intestinal (probably the gills).

Des truites arc-en-ciel de 3 g ont été exposés au Zn dissous dans l'eau et venant de régime semi-purifié. La vitesse de croissance et la concentration en ions du plasma ont été mesurées. A 16 semaines, les poissons exposés à une faible concentration en Zn (d'origine alimentaire et dissous dans l'eau) se sont révélés déficients en Zn telle que la montre leur faible vitesse de croissance et la faible concentration en Zn du plasma. Quand traité avec la quantité plus élevée, les poissons voient la concentration en Zn de leur plasma s'élever et leur vitesse de croissance s'accroître. Ceci suggère que le Zn peut être pris de deux façons possibles: par l'intestin ou par une autre voie, probablement les branchies.

CZ9.03

10)

THE EFFECTS OF ENVIRONMENTAL CHANGE ON THE GROWTH OF A FRESHWATER UNIONID CLAM, LAMPSILIS RADIATA. S.G.Hinch*, R.C.Bailey and R.H.Green, Ecology and Evolution Group, Zoology Department, University of Western Ontario, London, Ontario, Canada, N6A 5B7.

To examine the effects of habitat type on shell growth, freshwater unionid clams (Lampsilis radiata) were reciprocally transplanted between a wave-washed sandy site and an organic-rich mud site in Inner Long Point Bay Lake Erie. Growth rate analysis, using annual rings, showed that long-term growth in the sand was greater than that in the mud. A variable measuring overall size was derived from a principal components analysis of the lengths, widths and heights of the shells. This variable was analyzed in a factorial analysis of covariance of the transplant data, which revealed that differences in shell growth resulted from the original habitat of the clams rather than where they were transplanted. A genetic and environmental explanation for these results is presented, including a discussion of their relevance to unionid biomonitoring studies.

CZ9.04

1)

VARIATION IN MOLLUSCAN COMMUNITIES ALONG AN ENVIRONMENTAL GRADIENT. Robert C. Bailey, Ecology and Evolution Group, Zoology Dept., University of Western Ontario, London, Ontario, Canada. N6A 5B7

I examined the relationship between an environmental gradient (exposure) and the species richness of freshwater molluscs. The molluscs were collected at 41 sites in Inner Long Point Bay, Lake Erie. Three distinct guilds (filter-feeding mussels, deposit-feeding fingernail clams, and grazing gastropods) were present, so each guild was examined separately. A principal components analysis of environmental data from the 41 sites revealed a strong gradient of exposure (from mucky and protected to sandy and turbulent sites) in the bay. Only one of the guilds (the fingernail clams) showed a significant ($r=+0.63$, $p 0.001$) relationship between this exposure gradient and species richness. Two hypotheses (predator-induced diversity, community equilibrium gradient) which may explain this relationship (and the lack of one in the other two guilds) will be discussed.

Ecological Society of America, 71st Ann Mtg. Syracuse, New York. 1986.
decoupled from litter-derived nutrients as the surface soil dries, relying for production on N and P contributions from symbiotic associations, (1.2) while non-symbiotic plants (*Larrea tridentata*) cannot efficiently utilize deep soil water because of their inability to supplement subsoil nutrient supply. (2) In systems without deep water, symbionts act as parasites, consuming photosynthates, and decreasing productivity.

BAČIĆ, T. A. University of Split, 58000 Split, Yugoslavia. **Degree of xeromorphysmus of the leaves in some mediterranean plants on the basis of water content and water deficit.**

Content of water and water deficit and their fluctuations with amplitude of fluctuations have been investigated over a period of twelve months in the leaves of six evergreen mediterranean woody plants (*Quercus ilex* L., *Olea europaea* L., *Laurus nobilis* L., *Viburnum tinus* L., *Pittosporum tobira* Dryand. and *Nerium oleander* L.) and in the leaves of three mediterranean plants with leaves lasting several years (*Centaurea regusina* L., *Salvia officinalis* L. and *Inula candida* L.). It was observed that in all plants these two parameters are negatively correlated, and that this year's leaves have more water and less deficit than last year's ones. Also, from *Quercus ilex* to *Nerium oleander* (first group) and from *Centaurea ragusina* to *Inula candida* (second group) the content of water increases while deficit becomes smaller. Differences in investigated parameters between plants are the result of different degrees of leaf xeromorphic structure, while fluctuations are conditioned by strong influences of climatic factors.

BADGEROW, J. P. Syracuse University, Syracuse, NY 13210, USA. **Movement patterns of a freshwater gastropod in an artificial stream.**

An artificial stream was used to investigate the effects of controlled variation in substrate type, current velocity and light on movements of *Campeloma decisum*. Movements on sand and gravel were negligible due to a burrowing/sheltering habit. Movement on the exposed stream floor was continuous, allowing comparisons among the effects of current and light. Standing water resulted in essentially random movement in light and darkness. Moving water elicited a positively rheotactic response in most individuals. Rates of movement were similar at current velocities of 8 and 14 cm/sec and about 50% greater than in standing water. Light condition had no significant effect at the higher velocity. At the lower velocity, however, most snails traveled extensively upstream in the light but moderately downstream in the dark. These results suggest that although the purpose of the movement may be simple, the pattern reflects a complex response to interacting factors.

3)

BAILEY, R. C. University of Western Ontario, London, Ontario N6A 5B7, Canada. **Relationship between exposure and the shell growth and form of a freshwater mussel.**

I collected 240 *Lampsilis radiata* (Bivalvia; Unionidae) from nine sites in Inner Long Point Bay, Lake Erie. The length, width, height and weight of each mussel shell were measured. Sediment was also collected at each site, and the percent sand and loss on ignition were determined. Canonical correlation of the 'shell' variables with the 'substrate' variables indicated that larger clams were found at sites with sandier sediments (i.e., more exposed sites). Measurement of consecutive annual rings on each shell, followed by a Walford Plot analysis, also revealed faster growth rates in sandier areas. Length at one year of age, however, was greater in mussels from muckier areas, which is evidence of a trade-off between early and long-term growth. Faster growth by sand-dwelling clams is adaptive, since larger-shelled clams are more stable and better burrowers in an exposed, sandy habitat. Smaller shells prevent muck-dwelling clams from sinking in the substrate. Preliminary electrophoretic data show evidence of genetic differences in clams from the two habitats. A mechanism for the reproductive isolation of these two proposed ecotypes, given that there is a dispersal phase when the larvae are fish parasites, is proposed.

BAKER, D. B., J. W. KRAMER, and R. P. RICHARDS. Heidelberg College, Tiffin, OH 44883, USA. **Annual and seasonal variability in nutrient and sediment export from large row crop agricultural ecosystems.**

The Sandusky River, a northwest Ohio tributary to Lake Erie, drains 3240 km² of land area, 80% of which is used for row crop agriculture. Ten years of detailed studies reveal both the large magnitude and the extensive variability characteristic of nutrient and sediment export from large agroecosystems. Means and relative standard deviations for precipitation and discharge (cm/yr) and export of soluble phosphorus, total phosphorus, suspended solids and NO₃ + NO₂ - N (all kg/ha/yr⁻¹) were, respectively, 90.5 ± 7.5%, 35.0 ± 35.2%, 0.288 ± 39.7%, 1.62 ± 38.5%, 872 ± 52.3% and 16.0 ± 30.7%. January through March accounted for the following percentages of total precipitation or export: precipitation, 17.9%; discharge, 46.4%; soluble phosphorus, 52.5%; total phosphorus, 44.4%; suspended sediment, 38.4%; and NO₃ + NO₂ - N, 37.9%. The corresponding percentages for April through June were 31.2%, 27.3%, 20.7%, 35.5%, 44.8% and 37.0%. Research strategies for evaluating the effectiveness of agricultural pollution abatement programs must address the large annual variability associated with export from agroecosystems.

ABSTRACTS

5) VARIATION IN SHELL MORPHOLOGY AND GROWTH RATE OF THE UNIONID BIVALVE LAMPSILIS RADIATA: NATURE, NURTURE, OR NURTURED NATURE?

K. L. Bailey, Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7

Correlations between the shell morphology and growth rate of freshwater mussels and their environment have been described for over 70 years (eg. Ortmann's 'Law'). I measured such a correlation in Lampsilis radiata siligoidea from Inner Long Point Bay, Lake Erie. I then used allozyme data, heritability estimates, and a substrate preference experiment to form a hypothesis for the genetic mechanism causing the character variation. I also carried out burrowing experiments to test the adaptive hypotheses previously proposed for variation in shell morphology. Evidence from all of these studies suggests the presence of adaptive plasticity in these mussels.

THE RESPONSE OF PALYNOMORPHS TO PALEOENVIRONMENTAL CONDITIONS IN THE LOWER GULL RIVER FORMATION (MIDDLE ORDOVICIAN) OF SOUTHWESTERN ONTARIO.

Wendy D. Bunner and J.A. Legault
University of Waterloo, Waterloo, Ontario, N2L 3G1

The Gull River Formation in southwestern Ontario is a sequence of mudstones, wackestones and packstones which were deposited in a transgressing, tropical, epeiric sea during the Middle Ordovician. Through investigation of a continuous core from Kent County, covering roughly the lower forty percent of the formation, nine different lithofacies were delimited which represent deposition under various conditions within the shallow subtidal zone. Palynomorphs which were recovered from these sediments include twenty-eight species of acritarchs from thirteen genera, twenty species of Chitinozoa belonging to five genera, and a variety of scolecodonts. Paleoenvironmental conditions such as water energy, salinity and substrate type were reflected in the state of preservation, species distribution, diversity and relative abundances of acritarchs and Chitinozoa for each lithofacies.

SPONGES FROM THE CANADIAN ARCTIC

Tim de Freitas, Ottawa-Carleton Geoscience Centre, Department of Geology, University of Ottawa, Ottawa, Ontario. K1N 6N5

The Cape Phillips shales of the Canadian Arctic have yielded perhaps the most diverse suite of Silurian fossil sponges known anywhere. Sponge assemblages represent small but discrete opportunistic communities that, during the Silurian, nucleated on thin turbidite beds at the basin-platform

transition. Living in the stressful slope environment relatively free from competition with more effective space occupiers of the shallow water, the sponges proliferated to form thin biostromes. The communities were dominated by sponges (approximately 90 percent) with lesser amounts of algae, brachiopods, bryozoans, and crinoids. Sponges are of silica and well preserved, and are predominantly of one family, the Anthaspidellidae Miller: orchocladinid lithistids with a very distinctive ladder-like skeletal construction. Also in the collection is the first reported Silurian sphinctozoan sponge, Rigbyspongia catenula de Freitas. This sponge, in contrast to the lithistids, was constructed of aspicular calcium carbonate, but was subsequently replaced by silica. Other representatives include small acorn-sized subspherical sponges belonging to two different lithistid suborders: Tricranocladina Reid and Sphaerocladina Schrammen. Isolated hexactine spicules are ubiquitous, but only a few complete specimens, constructed of weakly fused hexactines and belonging to the class Hexactinellida Schmidt, are represented. A systematic study of the collection has shown that at least thirteen new species and five new genera are present.

CORALLINE ALGAL AND RELATED BIOFACIES OF THE RHODOLITHS IN THE GRENADINES BANK, LESSER ANTILLES ARC.

*DEY, Sarmistha and SMITH, Leigh, Department of Geological Sciences, Queen's University, Kingston, Ontario, K7L 3N6.

The crustose coralline algae are well-known frame builders in the fore-reef tropical waters (25 -100m) of the southern Grenadines Bank, Lesser Antilles island arc, east Caribbean.

The rhodoliths (red algal nodules) are comprised of coralline algae which produced crusts of different sizes, shapes, textures and mineralogies. The leafy crusts of the calcareous algae Archeolithothamnium, Neogoniolithon, Hydrolithon, Mesophyllum, Lithoporella and Lithophyllum are the dominant nodule builders. Besides coralline algae, other encrusting organisms as foraminifera (Homotrema, Gypsina), bryozoa (Steginoporella, Schizoporella) and sponge (Ceratoporella, Stromatospongia) act as secondary builders - forming smooth, warty or mammelated crusts.

The sequence of encrustations in the algal nodules is controlled by various ecological factors - as, depth of water, degree of agitation, salinity, temperature, intensity of light and nature of substrate. Selection of any encrusting organism forming the rhodolith also depends on competition, growth rate, turnover time and residence time of the nodule. Coralline algae is volumetrically important in nodule construction, because they compete for hard substrate either by growing faster or by overtopping their neighbours.

These ecological factors also control the growth forms of various epizoa and result in a wide variety of size, shape and internal structure of the rhodoliths.

The association/sequence of several encrusting organisms forming the rhodoliths can serve specific informations on the environmental, ecological conditions and hydrodynamic regime under which they have formed. This can also be utilized for reconstructing paleoecological conditions of ancient coated grains.

Canadian Paleontology and Biostratigraphy Seminar
Paleontology Division, Geological Society of Canada.
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7)

SHELL FORM AND EXPOSURE TO WAVE ENERGY IN THE
UNIONID BIVALVE ELLIPTIO COMPLANATA

R. H. Green, Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7

One hundred specimens of the unionid clam Elliptio complanata were collected from the shores of a small island in Baptiste Lake, near Algonquin Park. The windward shore is exposed to ca. 4 km. of fetch, whereas the leeward shore is close to high, nearby land. Shells of all specimens were measured, weighed and then sectioned for aging (by counting annual rings). A distinct pattern emerges, with faster growth rates being characteristic of the more exposed shoreline. This pattern supports results from most, though not all, other studies.

TOWARDS A REGIONAL MOLLUSCAN BIOSTRATIGRAPHY FOR THE LOWER UPPER CRETACEOUS OF PACIFIC COAST NORTH AMERICA

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The sequence of molluscan faunas in the Cenomanian to Coniacian portion of the Pacific coast Cretaceous is poorly understood, in contrast to older and younger parts of the system. However, recent field studies have provided important new data bearing on the molluscan biostratigraphy in this region.

In the Queen Charlotte Islands, Cenomanian strata have produced two faunas. The lower fauna comprises *Desmoceras* (*Pseudouligella*) *japonicum*, *Sciponoceras* cf. *baculoide*, *Graysonites* sp., *Puzosia* (*Anapuzosia*) cf. *dibleyi*, *Inoceramus* ex gr. *pictus*, and *I.* cf. *ginterensis* of early Cenomanian age and correlative with a similar, although more cosmopolitan, fauna in California. The upper fauna, of late Cenomanian age, includes *I.* aff. *incelebratus*, *Truncacilia haidana*, and *puzosi* ammonites. At present, a correlative late Cenomanian fauna has yet to be identified from California.

A three-fold division of the Turonian can be recognized in both California and British Columbia, with *Mytiloides labianus* indicative of the lowest part of the stage. The genera *Collignonoceras* and *Subprionocyclus*, diagnostic of the mid- and upper Turonian in California, have not yet been identified in western Canada; however, the mid-Turonian ammonite genus *Romaniceras* has been recognized in both regions and *I.* sp. cf. *multiformis*, representative of the late Turonian to early Coniacian, has been identified from the Queen Charlotte Islands.

The rich California Coniacian faunas include the Tethyan genera *Niceforoceras* and *Texasia*, as well as *I.* ex gr. *cordiformis*, none of which have yet been identified in Canada. However, mid-Coniacian *Peroniceras* and late Coniacian to early Santonian *Plesiotexanites* have been recognized in both California and British Columbia. Further field studies in these areas can only result in an increase in precision of correlation of mid-Cretaceous deposits throughout the entire Pacific region.

9)

WITHIN- AND AMONG-LAKE VARIATION IN SHELL MORPHOLOGY OF A FRESHWATER CLAM FROM SOUTH CENTRAL ONTARIO LAKES

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To compare the relative magnitude of variation in shell morphology within and among lakes, Elliptio complanata was collected from low and

high exposure areas in each of four small lakes in south-central Ontario. Nested ANOVA's on shell length, height, width and weight revealed that shell morphology varied much more between sites of differing exposure within a lake than among lakes of differing alkalinity. Canonical variates analysis showed that clams from high exposure areas had larger and proportionately taller and heavier shells than those from low exposure areas. There is no relationship between alkalinity of lakes and shell morphology. These results suggest that the use of unionid shell morphology to predict long-term whole lake water chemistry (e.g. alkalinity) requires sampling designs which take into account within-lake variation in shell morphology. Similar sampling strategies should be employed by those who wish to infer past environmental conditions from the clam fossil record.

A NEW APPROACH TO OLD PROBLEMS: SIMULATING MODERN AND ANCIENT SKELETAL METAZOAN COMMUNITY GROWTH BY MICROCOMPUTER

KOBLUK, David R., Geological Sciences, Erindale Campus, University of Toronto, Mississauga, Ontario, Canada L5L 1C6

Ancient invertebrate skeletal metazoan communities, such as reefs, have been best studied using 2 approaches: 1) observations and inferences derived by direct analysis of preserved community elements, and 2) by comparison with living analogs. Studies of living systems as analogs give clues to some information lost in ancient systems, such as organism-organism and organism-environment interactions, and soft-bodied elements, but provide little information on very long term community history and dynamics. Studying fossil systems gives a picture of the history of a community, but usually only limited reliable information on the dynamics of the community through time, and only glimpses of organism-organism and organism-environment interactions.

With the advent of powerful small computers, a third approach is now available: simulation of complex community dynamics over long spans of time. Biological and ecological simulations of various kinds have existed for some years, but they have not been applied to interpreting ancient communities, nor have they been able to track the history of the community simultaneously at all levels, from the individual to the whole system over geologically significant lengths of time.

A computer program is being developed that can simulate complex assemblages of organisms in real time, with many of the interactions that take place in a real world system, over long spans of time. The system currently runs on an Apple Macintosh Plus microcomputer, and is capable of simulating the interactive growth of an assemblage of thousands of hemispherical and tabular organisms representing up to 500 species for thousands of years. A much more powerful and complex version is being developed. The program-to-operator interface is very "user-friendly", and allows complete freedom to experiment with, and observe the effects of, combinations of controlling variables. The output is available in several forms, as tables of coordinates, areas, and summary statistics, graphs, and maps of the simulation surface with individual organisms drawn and coded. An important element of the current version of the program is an algorithm that incorporates a user-defined species competitive overgrowth hierarchy: at set-up time, the species are placed in an overgrowth hierarchy that in combination with growth rates, determines what will overgrow what when neighbors come into contact during each cycle of growth. Some of the other available options include storms, and control over larval settling rate.

This program is similar to others in being capable of complex functions, but differs from them in being able to track individuals in the system as well as the whole assemblage of organisms, for very long time spans, as well as provide graphic pictures of the simulation area with its community, at any time. The program has been written for a microcomputer, but has the processing power of some earlier (and some current) mini- and mainframe programs (although less speed). The flexibility of the user interface, ease of input of variables, as well as the incorporation of some complex algorithms that simulate important real world controls such as competitive hierarchies and storms, make this program and its successors a viable research tool. In its present form it already can be used to test some hypotheses of community dynamics, as well as observe how simple assemblages of organisms will interact and develop over long spans of time.

Ammonoculites, embryonic cephalopods and conodonts. This interpretation is not contradicted by the presence of serpulid worm tubes, and euryhaline ostracod and bivalve genera. Representatives of the exclusively fresh-water Arcellacea (Rhizopoda) have also been reported from certain horizons. They are represented by Diffugia-, Centrocyxis- and Helicopora-like forms.

The presence of these microfossils in the coal-bearing Sydney Mines Formation permits the positive determination of the environment of deposition, i.e. fresh-water or marginal-marine, and enables the detection of small-scale sea-level fluctuations within this formation. The distributions of the recent correspondents of these organisms, excluding conodonts, are essentially world wide, hence these forms may presumably occur in any Carboniferous deposit formed under similar environmental conditions.

CONODONT BIOSTRATIGRAPHY OF THE MIDDLE-LATE VISEAN WINDSOR GROUP IN BOREHOLE SH-1, STERWICK, NOVA SCOTIA by Peter H. von Bitter and Deborah P. A. Weston, Department of Invertebrate Palaeontology, Royal Ontario Museum and Department of Geology, University of Toronto, Toronto, Canada.

Three of the four conodont zones previously defined in the Windsor Group (Middle-Late Visean) were identified in borehole SH-1 at Sterwick, Nova Scotia. The lowest two, the Taphrognathus truncatelliticus and Clydagnathus visseogonius zones, are present in the lower Windsor Group and the uppermost, the Aptognathus scutellatus-Cavusgnathus Zone, is restricted to the upper Windsor Group. The lowest conodont zone, the Diplognathodus Zone was not identified in borehole SH-1.

The three conodont zones present can be related to previously established zones in the Windsor and Codroy groups of Atlantic Canada--thus aiding correlation within this region. The Taphrognathus truncatelliticus Zone correlates with the lower B macrofaunal Subzone and the lower portion of Miospore Zone NS. The Aptognathus scutellatus-Cavusgnathus Zone correlates with macrofaunal subzones C-K and Miospore Zone AT.

12) ANALYSIS FOR CONTAMINANTS IN BIVALVE MOLLUSC SHELLS-- A USEFUL APPROACH FOR POLLUTION STUDIES?

C. Walker, Department of Zoology, University of Western Ontario, London, Ontario N6A 3B7

Fresh water molluscs from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg) due to historic gold-mining activities in the area. Soft tissue concentrations of these elements vary among lakes, species and size classes, and are suspected to be influenced by age. To evaluate this influence and to compare longevities and growth rates among species and lakes, the shells of three species of mussels from two lakes were aged. Both external rings and shell sections were examined. Specimens of Elliptio complanata could be aged more accurately than either

12)

Anodonta imbecilis or Alasmodonta undulata. E. complanata grew more slowly, lived longer, and was significantly smaller at a given age at the most contaminated site.

Mussels accumulate contaminants in their shells as well as their soft tissues. Shells retain a sequential record of contamination in their annual growth rings, and therefore have potential for mapping changes over time. Two techniques for analyzing trace metals in growth rings, the electron microprobe and secondary ion mass spectrometry were evaluated. Both failed due to the lack of sensitivity and/or various interferences.

Results of studies of contaminants in shell for bivalves (freshwater and marine) from other contaminated sites are also discussed.

SEDIMENTOLOGY AND PRELIMINARY CONODONT BIOSTRATIGRAPHY OF THE UPPER DEVONIAN FAIRHOLME GROUP, NORDEGG AREA, WEST CENTRAL ALBERTA

J.A.W. Weissenberger, Department of Geology and Geophysics, University of Calgary, Calgary, Alberta T2N 1N4

The stratigraphic nomenclature of the Frasnian Fairholme Group is complicated by unresolved sedimentological and diagenetic problems. Solutions are proposed on the basis of facies analysis and conodont data from transects across the Cline Channel. The latter is a northeast-southwest trending basinal feature which separates the Fairholme and Southesk Cairn carbonate complexes.

The use of black chert in defining the Flume Member is questioned. The Cairn Formation-Peaches Member contact is diachronous, related to facies and diagenetic changes, while the Grotto and Arca Members are facies equivalents. The porous, light coloured platform margins at Hummingbird, Cripple Creek and Wapiabi Gap were initially 'drowned' by deeper-water coralline dolomites, not basinal shales. This coralline facies migrated landward during successive rises in sea level.

Two major sedimentary sequences, controlled by eustatic rises in sea level, are identified, along with numerous smaller depositional cycles. The first eustatic rise in sea level began in the Middle asymmetricus Zone. By Upper asymmetricus Zone, Perdreux Formation shales had overlain the lower Cairn Formation platform on both sides of the Cline Channel. Buildup and backstepping of platform margins continued to the end of the triangularis Zone. The second major eustatic cycle began in the glans Zone. This coincided with the beginning of Mt. Hawk Formation shale deposition. This unit represents the greatest extent of basinal deposition during the Frasnian.

A time-stratigraphy for the Frasnian carbonate complexes is defined by conodont dating, particularly of overlapping basinal units.

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